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PATHOLOGIC CHANGES IN GOUT

SURVEY OF ELEVEN NECROPSIED CASES *

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Renewed interest has been manifested in gout in recent years and, while much still remains to be clarified, the advances of the past decade are impressive. Insight has been gained into the basic nature of this disorder of purine metabolism and this has had practical application in more effective treatment. Among the more significant observations brought forth by Gutman and Yü,[1] Stetten,[2] Talbott,[3] Stecher *et al.*,[4] and others, mention should be made of elucidation of the hereditary factor in gout.

Especially noteworthy is the demonstration, through new isotope techniques, that hyperuricemia in some gouty subjects reflects an augmented, metabolically active pool of uric acid in the body, fed apparently by abnormal diversion of dietary glycine and other readily available metabolites to direct uric acid synthesis (uricotelic tendency). It also has been shown that in gout secondary to various hematopoietic disorders the turnover of nucleic acids involved in hemopoiesis is accelerated, with excessive formation of uric acid. Whatever the mechanism, accumulation of uric acid in these circumstances is of potentially serious consequence in man (as distinct from lower mammalian species) because of enzymatic inability to convert uric acid to allantoin and physiologic resorption by the renal tubules of fully 80 per cent of the glomerular urate filtrate. These factors, along with the relatively low solubility of uric acid and its salts in the tissue fluids, obviously favor the insidious formation of urate deposits in certain skeletal and extra-skeletal sites of predilection, which will be indicated presently.

With reference to improved therapeutic measures, one may cite the

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proved effectiveness of phenylbutazone and corticotrophin as well as colchicine in terminating attacks of acute gout, the use of regular colchicine prophylaxis to reduce the incidence and severity of acute episodes, and the concomitant administration of new uricosuric agents, such as probenecid, with a view to preventing chronic gouty arthritis and perhaps even ameliorating established deformities and disability.

Some definition of essential terms seems indicated in the interest of clarity. The word "tophus" has been used to denote hard deposits or concretions of varying types, even salivary calculi and pararticular syphilitic nodes. In this paper it will have reference only to localized, visible urate deposits, wherever they may be found. The term "gout" has also been used rather loosely in the past and the older literature contains references to so-called calcium gout (calcinosis), lipid gout (xanthoma tuberosum multiplex), and even oxalic gout (oxalism). The use of the designation gout in this vague miscellaneous sense is obviously confusing and is to be deprecated.

The present discussion is concerned exclusively with classical gout, which dates back to antiquity. This may be defined for basic orientation as a hereditary disorder of intermediary purine metabolism characterized by hyperuricemia, irregularly recurring attacks of acute arthritic involvement, and, eventually, a tendency to urate deposition, leading oftentimes to more or less severe, chronic tophaceous arthritis. For further clarification, it should be added that many persons (the relatives of gouty patients especially) may have a latent tendency to gout manifested only by otherwise unexplained hyperuricemia, without ever developing clinical gout. The latter is commonly ushered in by acute gout, affecting not only the first metatarsophalangeal joint (podagra) but frequently other joints as well.[5] The incidence and severity of these sporadic, excruciatingly painful attacks vary considerably from case to case and their pathologic basis is still a matter of conjecture. Between attacks (the so-called intercritical period) gouty patients may be ostensibly well. In fact, many never develop significant tophaceous gout. Some, however, after years of recurring episodes of acute gout, go on to develop chronic deforming changes associated with progressive urate deposition. While this is ordinarily a relatively slow, gradual process, it may be remarkably accelerated at times.[6] To round out the concept of gout and its sequelae, it is essential to note that in some patients there is an associated tendency toward vascular disease of potentially serious import, reflected in a significantly high incidence of hypertension, severe nephrosclerosis with renal insufficiency, cardiac failure, and cerebral vascular acci-

dents. The significance of these cardiovascular and renal changes, as observed in our material as well as by others, will be considered subsequently.

It would appear that general interest in gout on the part of pathologists has not kept pace with recent biochemical and clinical advances, perhaps for lack of opportunity to study relevant material, as Sherman[7] has suggested. Although gout is a fairly common disease, not a few pathologists still have difficulty in unequivocally identifying urate deposits microscopically through unfamiliarity with the distinctive pattern of foreign body giant cell reaction. Only a limited number of papers dealing with certain of the pathologic findings in gout, notably those of Bunim and McEwen,[8] Sherman,[7] Spitz *et al.*,[6] Kersley *et al.*,[9] and Traut and his associates[10] have appeared since 1940. It seems worth-while, therefore, to present in some detail a necropsied case of chronic deforming gouty arthritis of unusual severity, characterized further by remarkable calcification and ossification of tophaceous deposits. For collateral study, the protocols and slides of 10 additional necropsied cases of proved gout from our files, dating back to 1948, were reviewed.

The inferences culled from this material, amplified from the pertinent literature, will serve as a basis for comprehensive discussion.

Report of Case

The patient was a white male, 56 years of age, who was admitted to the hospital on April 19, 1952, because of severe deforming arthritis, as well as increasing weakness and periods of mental confusion. He was known to have had gout for about 35 years, first manifested by occasional episodes of acute gouty arthritis affecting his toes and/or fingers. These recurred sporadically and he went on to develop large tophi about the peripheral joints. By 1928 (at age 32) his fingers were no longer movable, and his feet were already severely deformed. During this entire period and throughout the rest of his life the patient observed no dietary restrictions and took colchicine only rarely and, then, in small ineffective doses. Despite his handicap, he remained active in business until 1944 (age 48), although he had been obliged to use crutches during the preceding 3 years. It is pertinent that no history of gout and/or hyperuricemia in other members of his family was elicited, although it appears doubtful whether thorough investigation was made.

By 1940 (at age 44) the effects of chronic tophaceous gout necessitated his seeking hospital treatment (elsewhere), the details of which are not available. He was first treated at this hospital in 1944 for progressive deforming arthritis and discharging tophi. At that time he presented slight hypertension (170/90 mm. of Hg), some impairment of renal function, and moderately severe secondary anemia. The serum uric acid level was noted to be 8.6 mg. per 100 cc.

The patient was observed here again in 1951, with manifestations of what appeared to be hypertensive encephalopathy. His blood pressure had risen to 230/135, and there were indications of increasing renal insufficiency. The uric acid level was estimated as high as 13 mg. per cent. After his cerebral symptoms

cleared in response to treatment, it was noted that the patient had sustained a fracture through the neck of the right femur, presumably during a convulsive seizure. This fracture failed to unite but open reduction was not attempted because of his poor renal status. Roentgen skeletal survey showed widespread, severe, gouty arthritis and striking radiopacity of the prominent tophaceous deposits, especially in the hands and feet, suggesting unusual calcification of them. The patient left the hospital, only to return after some months, when he suffered another attack of acute gout affecting the left knee.

During his terminal hospital stay, he was obviously in uremia and the blood urea nitrogen level rose steadily to 126 mg. per cent, while the uric acid level was reported as high as 14.1 mg. Despite supportive measures, he gradually lapsed into coma and died on the seventh hospital day.

Post-Mortem Examination

Gross Findings

The body was that of a somewhat obese white male, measuring 68 inches in height (Fig. 1) and weighing approximately 175 lbs. The hair was thin and graying. A number of small tophi, measuring up to 2.0 mm., were observed on the ears. The hands were strikingly deformed, presenting sausage-like enlargement of all the fingers from firm subcutaneous deposits, as well as marked flexion deformity of the left wrist (Fig. 2). There was also a marked flexion deformity of the left elbow. Over both forearms there were multiple, rubbery, freely movable, subcutaneous nodules, measuring up to 2.0 cm. in diameter (Fig. 4). The anterior abdominal wall was flabby, and the muscles of the extremities appeared wasted from disuse. Examination of the right lower extremity indicated the presence of a fracture of the femoral neck. Both knees appeared swollen. The feet were of twice the usual thickness, apparently from the presence of widespread, firm, tophaceous deposits, resembling those seen in the hands (Fig. 3). There were two sinuses over the right first metatarsophalangeal joint, apparently related to discharging tophi, and there were also a number of small scars on the hands and over the left knee.

The panniculus adiposus was 4 cm. in thickness and there was abundant fat in the omentum and mesentery. The liver extended 2.0 cm. below the right costal margin. Inspection of the abdomen showed nothing noteworthy, otherwise. The serous cavities contained no free fluid. The pleural surfaces were smooth and glistening.

The *heart* weighed 375 gm. and the pericardial sac was rather fatty. The noteworthy changes were dilatation of the right auricle and ventricle, moderate hypertrophy of the left ventricle, and calcification of the mitral ring, as well as of one of the aortic cusps. The coronary arteries were thin-walled and widely patent, while the aorta showed relatively little atheromatous change.

The *thyroid gland* showed nothing unusual.

Three *parathyroid glands* of normal size were identified.

Lungs. The tracheobronchial tree contained a large amount of viscid brown sputum and the mucosa of the larger bronchi appeared hyperemic. The lungs weighed 800 gm. each and, on section, showed slight congestion and edema.

The *liver* weighed 2,100 gm. and its surface was yellow-brown, smooth, and glistening. Its architecture appeared unaltered.

The *gallbladder* contained 10 cc. of brown bile and presented a thickened wall, in which there were focal submucosal deposits of calcareous gravel. The bile ducts were not remarkable.

The *spleen* weighed 150 gm. and presented a firm, reddish purple pulp.

The *pancreas* was heavily infiltrated by fat, but was not unusual otherwise.

The *adrenal glands* showed thin cortices, somewhat depleted of lipid.

The *kidneys* were appreciably reduced in size and presented irregular, coarsely granular cortical surfaces, pitted by retention cysts, ranging in size up to 1.5 cm. in diameter. There were also a number of small yellow-white, slightly elevated nodules resembling cortical adenomas. On section, the kidneys were pale gray-brown and the markings of cortex and medulla could not be clearly distinguished (Fig. 21). Also noted were several chalky, yellow-white deposits within the medulla, resembling tophaceous material. The pelves and calyces appeared slightly dilated and thickened, and contained creamy yellowish fluid. The ureters also appeared thick-walled, but were of normal caliber.

The *urinary bladder* was thickened and trabeculated, and contained creamy, inspissated urine. Its mucosa appeared hyperemic and somewhat edematous. The *prostate* was nodular and contained tiny calcific concretions. The *seminal vesicles* appeared normal, as did the testes.

The *gastro-intestinal tract* showed nothing noteworthy.

Brain. The calvarium, dura, and leptomeninges were not unusual. The brain weighed 1,400 gm. and, on serial coronal section, presented no gross abnormalities. The vessels at the base were thin-walled and patent.

Skeletal System. Inspection of a ventral slice of the lower dorsal and lumbar vertebral column showed chalky deposits within the intervertebral disks and the contiguous spongy bone; the bodies, otherwise, were not altered (Fig. 11).

Examination of the right hip joint showed a rent in the capsule, allowing thick, creamy, blood-stained material to exude into the adjacent muscles and fascial planes. The acetabulum was extensively modified. The head of the femur constituted a loose fracture fragment; its articular surface was partially denuded and irregularly coated by yellow-white urate deposits suggesting drops of paint. There was non-union at the fracture site in the femoral neck, and at the bone ends one observed fibrous tissue coated by a creamy white paste (Fig. 12). Chemical assay of this material indicated a concentration of calcium as high as 4.0 per cent and of urate, exceeding 11.0 per cent.

Inspection of the left knee joint disclosed a thin, irregularly dispersed spattering of chalky white material on the articular surfaces of the femur and tibia. This was most prominent around the edges of the articular bone ends and was generally absent at contact points. The cartilage was almost completely destroyed. The articular surface of the patella was likewise extensively denuded of cartilage and coated by heavy deposits of whitish urate material. Sections through the articular bone ends revealed several, comparatively soft, chalky white deposits within the femur and tibia, just beneath their articular ends. Within the lower end of the femur, some 4 cm. proximal to its articular surface, there were additional localized urate deposits surrounded by fibrous tissue and apparently condensed spongiosa (Fig. 9). The synovial membrane of the knee joint showed villous hypertrophy in places, as well as chalky granular deposits in the vicinity of the patellar ligament (Fig. 10). Within the ligament also, there were a number of similar foci ranging up to 1.0 cm. in greatest diameter.

The feet were remarkably swollen and deformed, as noted (Fig. 3). At the level of the head of the third metatarsal, they measured as much as 6 cm. in their supero-inferior dimension. Beneath the skin and extending to the modified atrophic remnants of the foot bones, there was a thick layer of gray-yellow, granular, calcareous material and chalky firmer deposits, interspersed by tracts of fibrous tissue (Fig. 13). In the perionychial spaces also, there was a layer of chalky material extending into the nail beds. The right foot was sectioned sagittally in the plane of the middle ray. On section, the head of the third metatarsal bone presented as a thin shell containing pinkish gray mucoid material and irregular deposits of chalky white paste. The discernible phalanges appeared to be partially destroyed and similarly replaced by chalky deposits. The interphalangeal joint spaces, too, appeared virtually obliterated by calcareously impregnated urate deposits.

Microscopic Findings

The significant findings have been incorporated in the anatomical diagnosis and will not be described in detail here with exception of those relating to the manifestations of gout and its sequelae.

Skeletal Tissues. Sections of the foot showed the presence in the deeper layers of the skin and subcutis, as well as in the underlying fascia, of extensive, conglomerate, smaller and larger urate deposits. These were ringed in characteristic fashion by foreign body giant cells, as well as mononuclear histiocytes (Fig. 15), and presented, as an added feature, prominent and often heavy calcium deposition within urate material. Also in evidence were trabeculae of reactive new bone around the periphery of some of the urate deposits (Figs. 16 and 17). The latter appeared amorphous for the most part, and only in the alcohol-fixed preparations could one discern crystalline structure. The connective tissue between the focally calcified urate deposits contained fibroblasts, adventitial reticular cells mobilizing as macrophages, and a scattering of small mononuclear cells.

Sections of the femoral head (constituting the proximal fracture fragment) showed the articular cartilage to be eroded in places and replaced by urate-containing pannus. Urate deposits were present also within the subchondral bone and marrow. As noted grossly, there were prominent urate deposits at the site of pseudo-arthrosis in the femoral neck (Fig. 12), as well as numerous polymorphonuclear leukocytes indicative of localized infection.

Sections of the knee joint capsule showed pronounced villous hypertrophy of the synovium and focal deposits of urate material within the synovial lining and subjacent tissue. Here, too, calcification of urate deposits was a conspicuous feature (Fig. 19).

Sections of the vertebral column showed conglomerate urate deposits within the intervertebral disk tissue, as well as in the contiguous spongiosa of the bodies. Within the vertebrae, at sites of urate deposition, were observed interstitial fibrosis and slight reactive osteosclerosis.

Sections of both *kidneys* showed widespread cortical scarring and interstitial inflammation, apparently indicative of chronic pyelonephritis. There were also polymorphonuclear leukocytes within the areas of fibrosis, as well as in some of the tubules. Also noted were partial or complete obliteration of many glomeruli, dilation of tubules, and moderate thickening and narrowing of the small arterial branches. Multiple small cortical adenomas were dispersed through both kidneys. Within the tubules there were many small calcific concretions. Deep

in the parenchyma there were a number of spaces (without distinct lining) which contained urate material admixed with calcium. There was leukocytic reaction about them, as well as in the surrounding interstitial connective tissue. The latter also contained occasional small, focal, urate deposits ringed by small histiocytes. The renal pelves showed chronic inflammation of the fat tissue and mucosa, which also presented focal erosions.

Sections of the *prostate* showed fibro-adenomatous hyperplasia and chronic inflammation of the larger ducts. Many of the smaller ducts and acini contained inspissated secretion and within the lumina in one group of dilated ducts there were yellow-brown needle-like crystals resembling those of urate deposits. They were surrounded by foreign body giant cells and were associated also with intense focal calcium deposition (Fig. 23).

While the *parathyroid glands* were not appreciably enlarged, they showed significant chief-cell hyperplasia, apparently reflecting long-standing chronic renal insufficiency.

The remaining sections showed nothing remarkable, except as indicated in the anatomical diagnosis.

Anatomical Diagnosis

Far advanced, chronic tophaceous gout: chronic gouty arthritis of long standing, involving almost all joints, with severe deformity; extensive subcutaneous tophi in hands, feet, forearms, ears, with pronounced calcification and regional heterotopic ossification; urate deposits within fascia, tendons, ligaments, periosteum, articular bone ends, at fracture site (femoral neck), and in intervertebral disks; urate deposits within renal tubules; prostatic concretions, apparently containing urates. Severe chronic and acute pyelonephritis, with extensive alteration of renal architecture; uremia; hypertensive encephalopathy (clinical); old fracture of right femoral neck, with non-union; generalized osteoporosis; chief-cell hyperplasia of parathyroid glands (microscopic). Generalized arteriosclerosis; lipomatosis and intralobular fibrosis of pancreas; cholelithiasis.

Discussion

Hereditary Character of Gout

The familial incidence of gout has long been recognized. Recent investigations[11,12,4] of the pedigrees of numerous gouty families have shown that many members apparently free of symptomatic gout nevertheless manifest hyperuricemia. If one accepts the reasonable premise that hyperuricemia in these circumstances represents an expression

of latent gout, it becomes possible to study the full genetic pattern of familial inheritance. According to Stecher, Hersh, and Solomon,[4] the available data strongly suggest that the tendency to gout is transmitted as an autosomal dominant genotype having a much lower penetrance in the female than in the male (approximately 1:20). The *modus operandi* of this genetic defect in biochemical terms is still obscure and its clarification would seem to hinge upon better understanding of the essential enzyme reactions in the intermediary metabolism of purine derivatives, a field which is now being explored profitably.[13]

The Nature of Acute Gout

There has been much speculation in regard to the pathogenesis of acute gout. However, apart from a keener awareness of numerous precipitating "stress" factors, we have no better insight actually than did Sydenham[14] in 1683, when he rendered his classical description of the torture he endured during gouty attacks. For that matter, the basis for the specific action of colchicine in alleviating these attacks is still not understood, although the drug has been in use for some 1400 years. It is quite conceivable that elucidation of the biochemical action of this remarkable alkaloid (used also in plant breeding and genetics) may yet furnish the key to solution of the problem. As for direct pathologic observation, according to Talbott[3] not a single report of the gross or microscopic examination of the interior of an affected joint during an acute attack has appeared, apparently because no one has had the temerity to secure material for biopsy in these circumstances. Nor has the condition ever been reproduced or simulated experimentally by any means whatscever. For reasons which have been cogently marshalled elsewhere,[15] urate deposition *per se* cannot be held responsible plausibly for acute gouty attacks. As Gutman[16] has tentatively suggested, however, it is possible that acute gout is provoked by some precursor of uric acid, perhaps an intermediary purine metabolite as yet unidentified. Although the concept also has been advanced[17] that temporary adrenocortical deficiency may be the trigger mechanism, the observations of Levin, Rivo, and Bassett[18] would seem to cast serious doubt upon the soundness of this view. In any event, this factor in itself would hardly account altogether for the acute gouty episode.

Urate Deposition in Chronic Gout

In contrast to acute gout, there is every indication that chronic gout results from very gradual, but appreciable deposition of urates, especially within and about the joints, and that no other essential etiologic

factors need be invoked. Even in birds (which, unlike man, are well equipped to dispose of uric acid as the normal nitrogenous waste product of ordinary protein, as well as of purine catabolism), a condition resembling chronic tophaceous gout, so-called avian gout, may be induced experimentally by prolonged forced feeding of protein, by ligation of both ureters, or by the use of renal poisons. Under these conditions, as Bauer and Klemperer[19] have cited, urate deposition takes place in the joint cartilages and large tophi appear in the extremities.

Fortunately, according to Talbott[3] and other experienced clinicians, chronic deforming gouty arthritis is the ultimate fate of only a limited number of patients afflicted with gout and particularly of those who suffer frequent attacks of acute gout while still comparatively young. While most patients with gout are middle-aged when they first experience arthritic attacks, the latter may occur in younger persons, and have been noted even in children. Incidentally, the subject in the case reported sustained his initial bout of acute gout at age 21, and at the comparatively early age of 32 already presented severe deformity and limitation of motion of his hands and feet. His prolonged neglect of prophylactic treatment may well have contributed to the extensiveness of the urate deposits observed at necropsy.

In chronic tophaceous gout the characteristic tissue response to urate deposits, wherever they may be encountered, is essentially that of a peculiar foreign-body reaction (Fig. 15). As noted, the urate material usually appears rather amorphous in formalin-fixed specimens, and only in alcohol-fixed preparations does one regularly discern crystalline structure. This apparently depends upon the presence of sodium biurate,[9] with an admixture also of protein and, occasionally, of a small quantity of cholesterol. On microscopic examination, as indicated, one characteristically observes smaller or larger, discrete or conglomerate deposits, ringed peripherally by foreign body giant cells and/or mononuclear histiocytes. This reaction pattern is sufficiently distinctive, so that blackening with silver[20] is scarcely required for confirmation. The inflammatory response otherwise usually is rather inconspicuous provided that there has not been ulceration or complicating infection, although one may note slight interstitial fibrosis and occasional macrophages or small mononuclear cells.

The finding of striking widespread calcification of urate deposits in our major case merits brief comment. Localized calcification of tophi in gout, while noteworthy, is apparently not too uncommon. A number of writers[21,22] have remarked, for example, that sizeable tophi

of long standing in olecranon or prepatellar bursae may occasionally become sufficiently impregnated with calcium to render them radiopaque. Also, in one of our other cases tophaceous deposits in a toe were found to be calcified. Massive widespread calcification of tophaceous material, however, appears to be distinctly unusual. In the necropsied case of gout reported by Kersley, Mandel, and Jeffrey,[9] pronounced calcification in the tophaceous soft parts of the hand was demonstrated. Similarly, Talbott[3] illustrated heavy calcium deposits associated with urate tophi within the bones and soft parts of the foot, although he referred to the observation as being almost unique. By the same token, the finding of conspicuous heterotopic ossification within tophaceous deposits in our case (Figs. 16 and 17) likewise is remarkable, and it may well be that the presence of abundant calcium was a significant predisposing factor.

Calcification of urate deposits seems clearly to represent a secondary change in point of time. Furthermore, in our case at least, it did not appear to be an expression of secondary hyperparathyroidism associated with chronic renal insufficiency. It is true that the parathyroid glands showed hyperplasia microscopically. On the other hand, the evidence of skeletal resorption apart from disuse atrophy was minimal, and appreciable calcium deposition was observed only within tophaceous material.

Skeletal Alterations. That the skeletal connective tissues, particularly the joints and periarticular structures, bear the brunt of urate deposition in tophaceous gout is common knowledge. For a detailed account of these skeletal alterations one may turn to the article by Lang,[23] which is useful also for its compilation of the pertinent German literature. It may be worth-while, however, to emphasize the essential changes for convenient orientation. Within an affected joint (be it a metatarsophalangeal, metacarpophalangeal, or interphalangeal joint, a knee, a hip, a shoulder, or any other) focal, chalky white or yellow, tophaceous deposits are observed on the articular surfaces (appearing oftentimes as though painted). They are also found in the deeper layers of the cartilage. The articular cartilage tends gradually to be destroyed and replaced by pannus (Fig. 20). Eventually, in severe instances with attendant deformity and immobilization, fibrous ankylosis may ensue,[24] as well as the usual changes of secondary osteo-arthritis. The latter are often reflected in the appearance of roentgenographically discernible subchondral rarefactions, although these in themselves are by no means pathognomonic of gout, as is sometimes assumed. Concomitantly, the synovial lining and the sub-

lining connective tissue of the affected joint capsule likewise exhibit focal impregnation by urates and, eventually, more or less extensive, reactive, chronic villous synovitis ensues (Fig. 19). The articular bone ends frequently manifest urate deposits within their periosteal covering, as well as in the subchondral spongy bone (Figs. 9 and 18). The ligaments and tendons, too, may be more or less heavily impregnated (Figs. 16 and 17). Similarly, certain of the pararticular bursae, especially the olecranon and prepatellar bursae, are often predilected. In the vertebral column, as seen in our case, the intervertebral disks and the contiguous portions of the bodies may be the sites of appreciable urate deposition (Fig. 11). In the noteworthy case meticulously recorded by Kersley and his associates,[9] tophaceous involvement of the first cervical vertebra (among other sites) was so extensive as to bring about subluxation of the upper cervical spine and impending compression of the cord.

Extraskeletal Urate Deposits. As noted by numerous investigators,[25,26] the deposition of urates in gouty subjects may be encountered in certain extraskeletal sites as well, with greater or lesser frequency. Tophaceous involvement of the skin and subcutis, especially of the hands and feet and occasionally of the ear, is, of course, commonly observed in instances of moderate severity. Involvement of the eyelids, the cornea, and the sclera has also been noted, although this is comparatively unusual and we have not observed it in our own material. More important, urate deposits frequently are encountered at necropsy in the kidneys and occasionally in the lower urinary tract as stones or gravel. Also noteworthy is deposition in the heart and blood vessels, observations of which will be cited in the following section. The literature also contains casual reference[25] to the finding of urate deposits within the cartilages of the upper and lower airways (nose, epiglottis, vocal cords, arytenoid cartilages, and the bronchi), as well as in other rare sites, specifically the tongue, prepuce and corpus cavernosus of the penis, testis, pleura, and meninges. These latter references (which we were unable to verify) are to be found mainly in the older German literature and date back to a period when inordinately severe, tophaceous gout apparently was more common than it is today. Among the findings mentioned, the cardiovascular and renal changes are of sufficient interest to merit further consideration.

Cardiovascular Lesions. Specific involvement of the heart in tophaceous gout is comparatively rare and was not noted in our material, although there are a limited number of pertinent observations on

record. The finding of urate deposits in the pericardium has been cited by Kaufmann.[26] With reference to myocardial involvement, Hench and Darnall[27] have called attention to a remarkable case in which complete heart block was caused by a large (urate) tophus affecting the conduction bundle. Also noteworthy is the observation by Bunim and McEwen[8] of urate deposition in the posterior leaflet of the mitral valve of a 63-year-old man with long-standing, chronic tophaceous gout. This tophus was sharply circumscribed, about 4.0 cm. long and 0.5 cm. thick, and was associated with little, if any, inflammatory change. In this connection, mention should be made of another pertinent instance recently described by Traut and his associates[10] in which urate deposition was noted likewise in a mitral valve leaflet extending down over the endocardium of the left ventricle, and apparently in the aortic cusps as well. The same authors reported another instance of gout in which urate crystals were believed to be present within the wall of a coronary artery and within several of the intra-abdominal arteries. Positive identification was lacking, however, and the possibility that the crystals observed were of the nature of cholesterol cannot be dismissed. In surveying our own 11 necropsied cases of gout, careful search was made for urate deposits within blood vessels, but none was found.

Renal Changes in Gout. It has been stated[3] with apparent justification that renal failure is the single most important cause of death in gouty patients irrespective of age, and the only important cause of premature death. It is noteworthy that the primary cause of death was uremia in 5 of the 11 necropsied cases of proved gout studied by us. All but one of these patients were men in their fifties. This experience, while limited, is essentially in accord with the clinical observations of Schnitker and Richter[28] on 55 patients with gout, 17 of whom (approximately one third) presented evidence of renal insufficiency. As Talbott[29] has indicated, chronic renal impairment need not necessarily go hand in hand with advanced joint disease and may, in fact, completely overshadow what appears clinically to be minimal arthritic involvement. In any event, once renal insufficiency develops, it tends to aggravate the manifestations of tophaceous gout through uric acid retention and further elevation of the plasma urate.

Collaterally, another serious hazard of many gouty patients apparently is their tendency to develop hypertension and significant arteriosclerosis, for reasons which are not yet altogether clear. This trend also is reflected in our material in that another 5 of the 11 patients succumbed to coronary thrombosis, congestive heart failure, or intra-

cranial hemorrhage. These findings are in harmony with those of Brown and Mallory,[30] among others. Although Fishberg[31] has expressed the view that there is no convincing evidence to indicate that gout plays a rôle in the pathogenesis of essential hypertension, their positive association cannot be dismissed readily as fortuitous. Specifically, the systolic and diastolic blood pressures were noted to be moderately or markedly elevated in 7 of our 11 gouty subjects. The corresponding weights of these hearts at necropsy ranged from 450 to 650 gm., with the majority about 500 gm. While the number of cases surveyed is not highly significant statistically, it is perhaps noteworthy that all but one of the gouty subjects necropsied here within the past several years died of cardiovascular or renal complications.

The concept of "gouty nephritis" or of "gout kidney" has long been a rather vague one[32] and it seems questionable whether it is advantageous to retain these old designations, except perhaps for convenient reference to the specific instances in which appreciable urate deposition is demonstrated in the renal parenchyma. The general opinion[30,33] at present is that the kidneys of gouty patients suffering from renal insufficiency present a variety of changes characterized by more or less severe arteriosclerotic contraction, chronic pyelonephritis, and frequently, though not invariably, the presence of urate deposits. As Brown and Mallory[30] have emphasized, the latter are encountered mainly within the lumina of collecting tubules and in their vicinity, where they tend to obstruct, to destroy the tubular epithelium and, in general, to set up a focus of subacute or chronic pyelonephritis. Occasionally, the presence of uric acid stones or gravel in the renal pelvis may be another predisposing factor favoring the development of pyelonephritis, although this is not very common and was observed in only one of our cases. On the other hand, urate deposition within the renal pyramids and associated with significant pyelonephritis was noted in 4 of the 5 cases terminating in uremia. When these urate deposits are comparatively small and surrounded by small macrophages rather than conspicuous foreign body giant cells, their significance may be readily overlooked by an inexperienced observer.

Summary

This paper deals with the pathologic changes in gout, as indicated by the findings in 11 necropsied cases and by collateral study of the pertinent literature. A detailed account is given of the findings in one of these cases, an instance of chronic deforming gouty arthritis of unusual severity, characterized by remarkable calcification and

ossification of tophaceous deposits. While emphasis has been placed upon the pathologic changes observed in fully developed chronic tophaceous gout, particularly the skeletal, cardiovascular, and renal alterations, an attempt has been made also to bring the condition as a whole into sharper focus in the light of recent clinical and biochemical advances.

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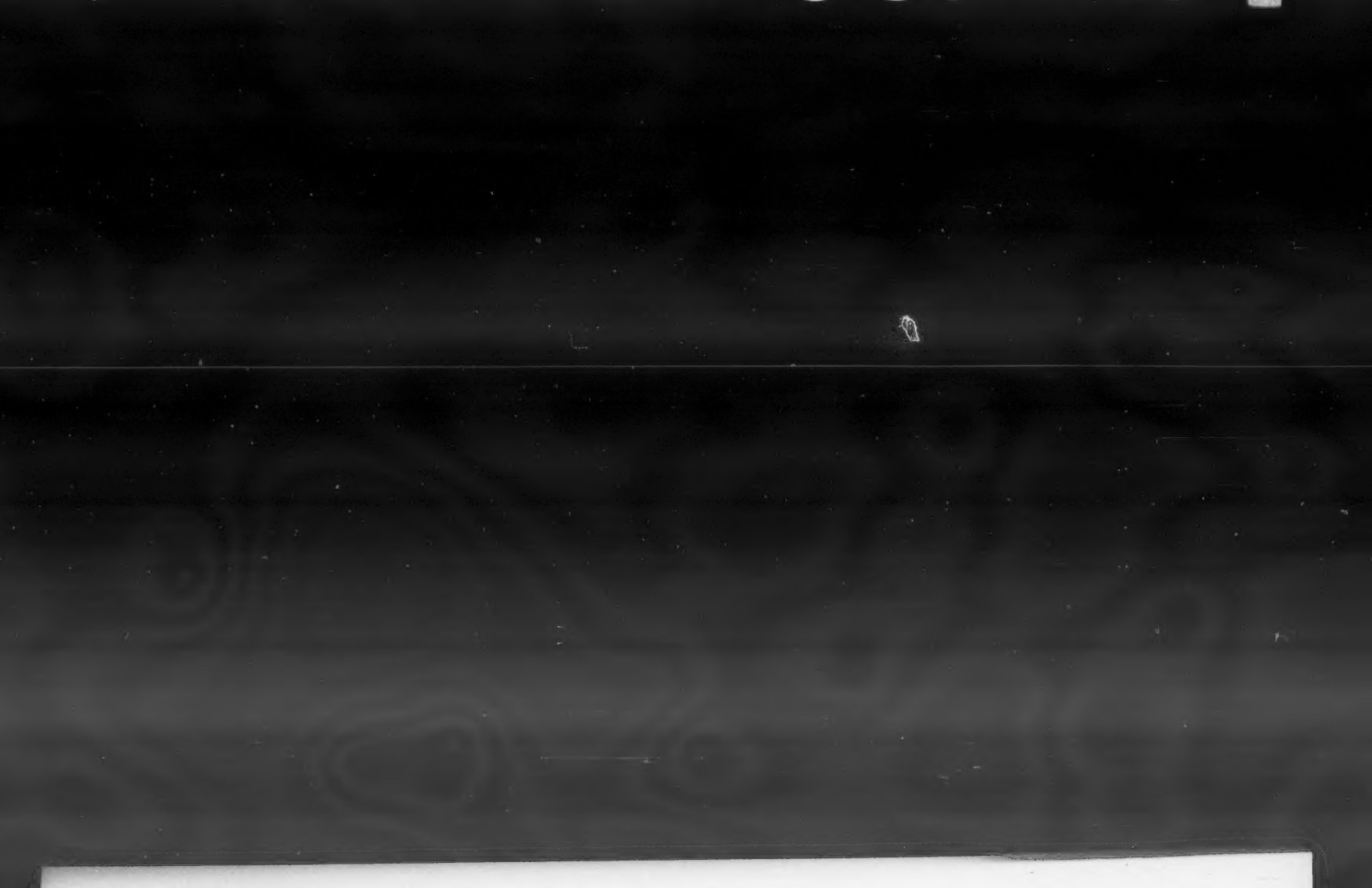
LEGENDS FOR FIGURES

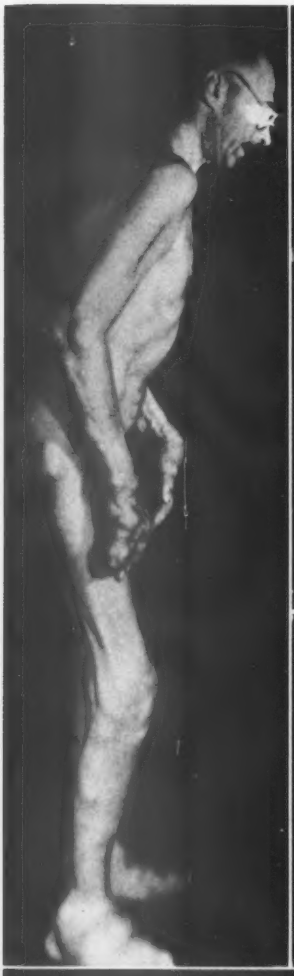
FIG. 1. The patient in the case reported in detail, showing widespread prominent tophi and the effects of deforming arthritis.

FIG. 2. A hand of this patient, showing flexion deformity at the wrist and sausage-like expansion and deformity of the fingers, reflecting extensive tophaceous deposits. This may be compared with the roentgenogram in Figure 7.

FIG. 3. Comparable changes in the foot of this patient, illustrated also in Figure 8.

FIG. 4. Multiple tophi in the elbow region and forearm.





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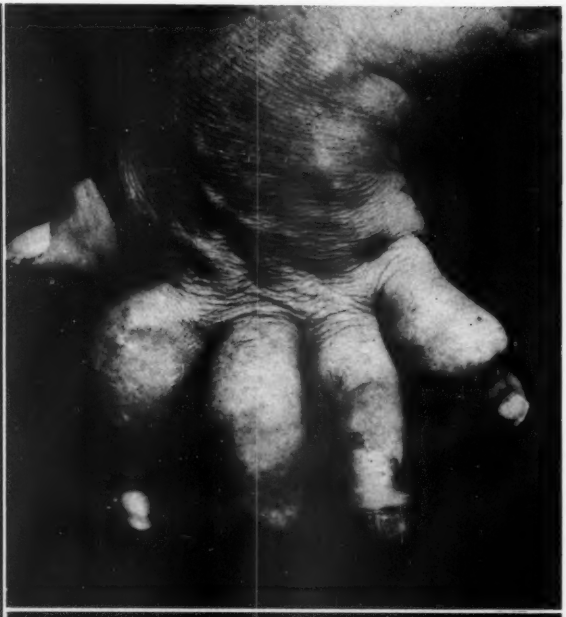

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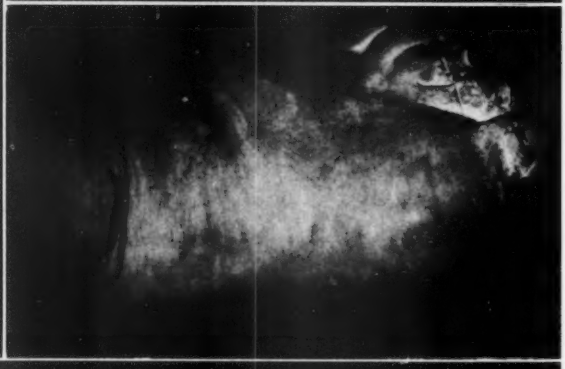

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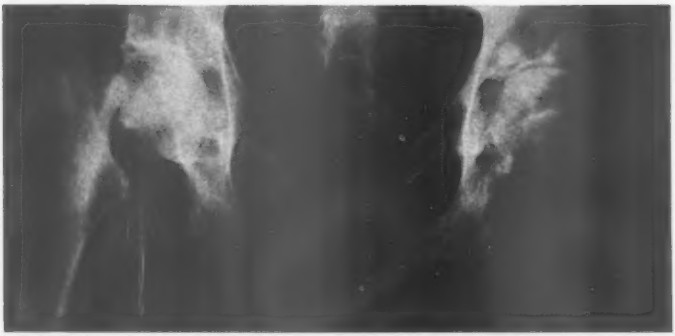

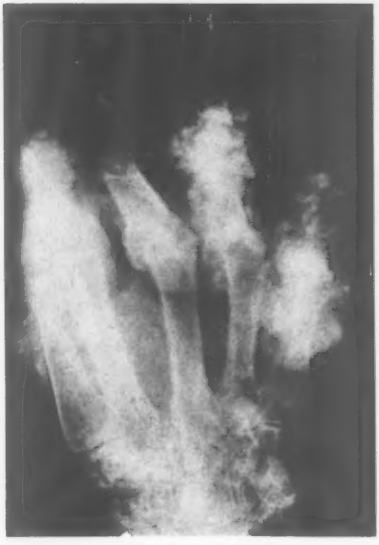

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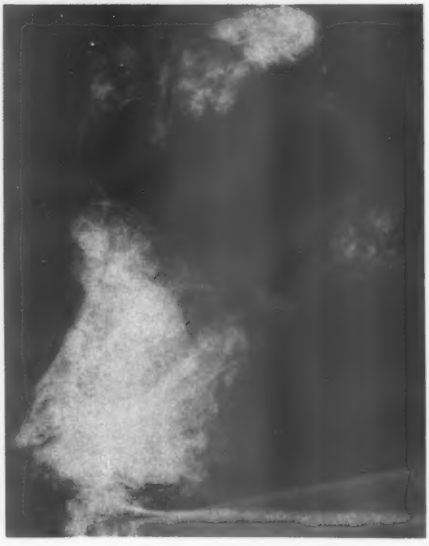

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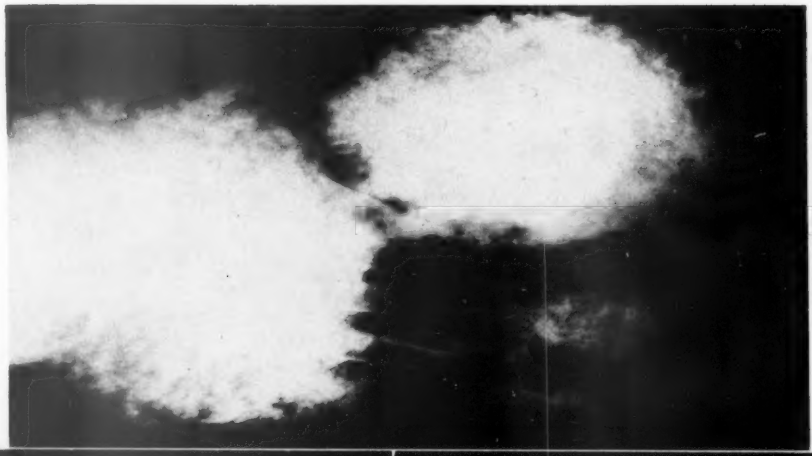
FIG. 5. Roentgenogram of the pelvis and upper femora in the case illustrated in Figures 1 to 4, showing radiopaque tophaceous deposits in the hips and ischial tuberosities, and an un-united fracture in the neck of the right femur.

FIGS. 6 and 7. Roentgenograms of the hands in the same case, showing striking radiopacity of tophaceous deposits, reflecting heavy calcification. Of note also are the skeletal alterations associated with advanced chronic gouty arthritis.

FIG. 8. Comparable changes to those in the hands are shown in a foot of the same patient.



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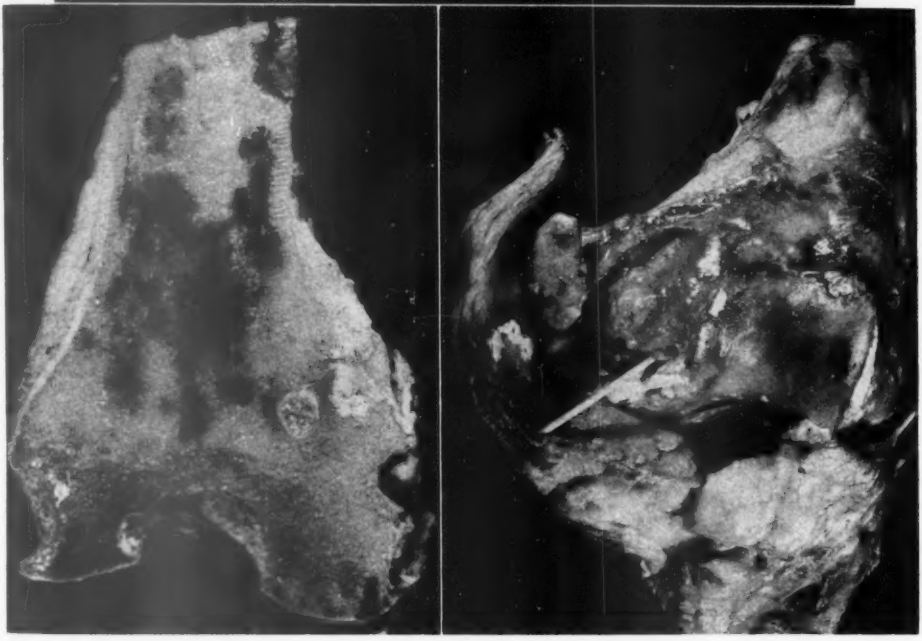

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FIG. 9. A frontal section of the lower femur in the necropsied case reported in detail, showing focal urate deposits subchondrally and also deep to the joint surface. See also Figure 18.

FIG. 10. Photograph of the lining surface of the capsule of the knee joint, showing chalky (yellow-white) flecks of urate deposit within the synovial membrane and on the articular cartilage of the patella, especially around its periphery. This may be compared with Figures 19 and 20.

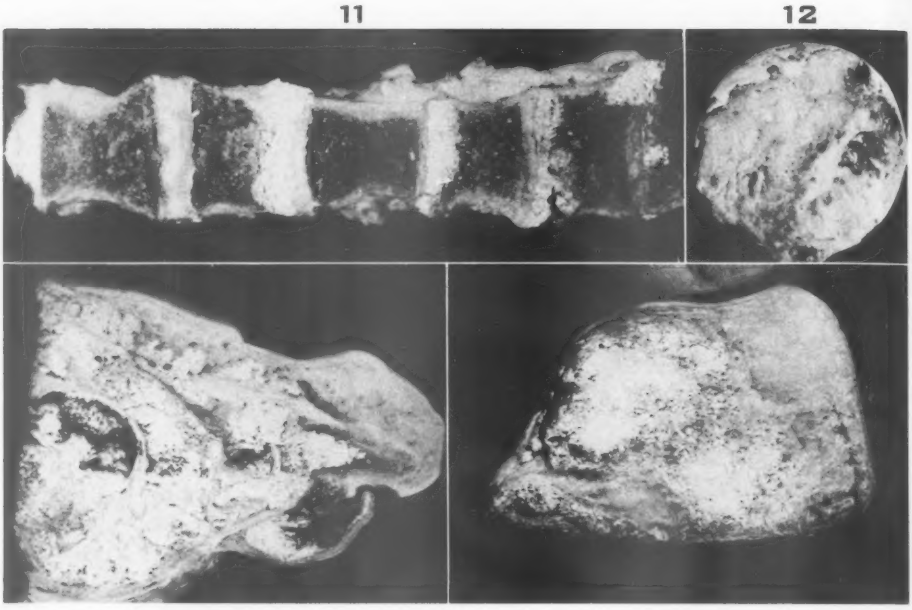

FIG. 11. Ventral slice of a segment of the vertebral column showing heavy urate deposits within most of the intervertebral disks and extending into the contiguous bodies.

FIG. 12. The under surface of the femoral head (constituting a proximal fracture fragment) heavily coated in places by a streaky layer of urate deposit. This site was bathed in a thick, white, creamy paste rich in calcium and in urates (determined by chemical assay).

FIG. 13. Sagittal section through the middle ray of the right foot demonstrating substantial destruction of the bones and diffuse deposition of chalky urate material. Sections showed the latter to be calcified and ossified in places. See Figure 17.

FIG. 14. Another field comparable to that of Figure 13, and likewise showing abundant calcified tophaceous deposits.

FIG. 15. A representative field, showing urate deposits characteristically ringed by foreign body giant cells, as well as smaller macrophages. This block was fixed in formalin, rather than alcohol, and crystalline structure is lacking. × 250.

FIG. 16. Conglomerate urate deposits within the plantar fascia. There is a tendency to new bone formation at the periphery of some of these deposits. × 55.

FIG. 17. Calcification and appreciable ossification of tophaceous material within the skin and subcutis of a foot. × 55.

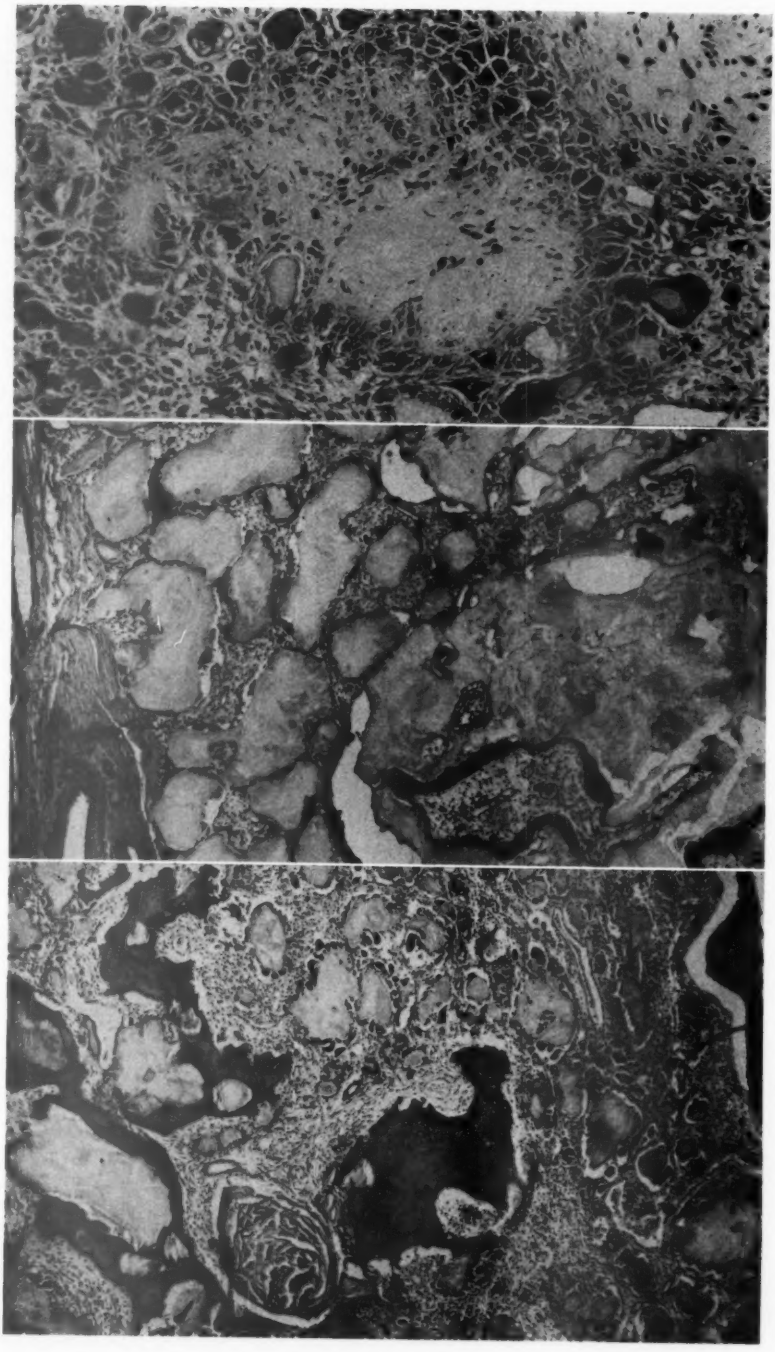

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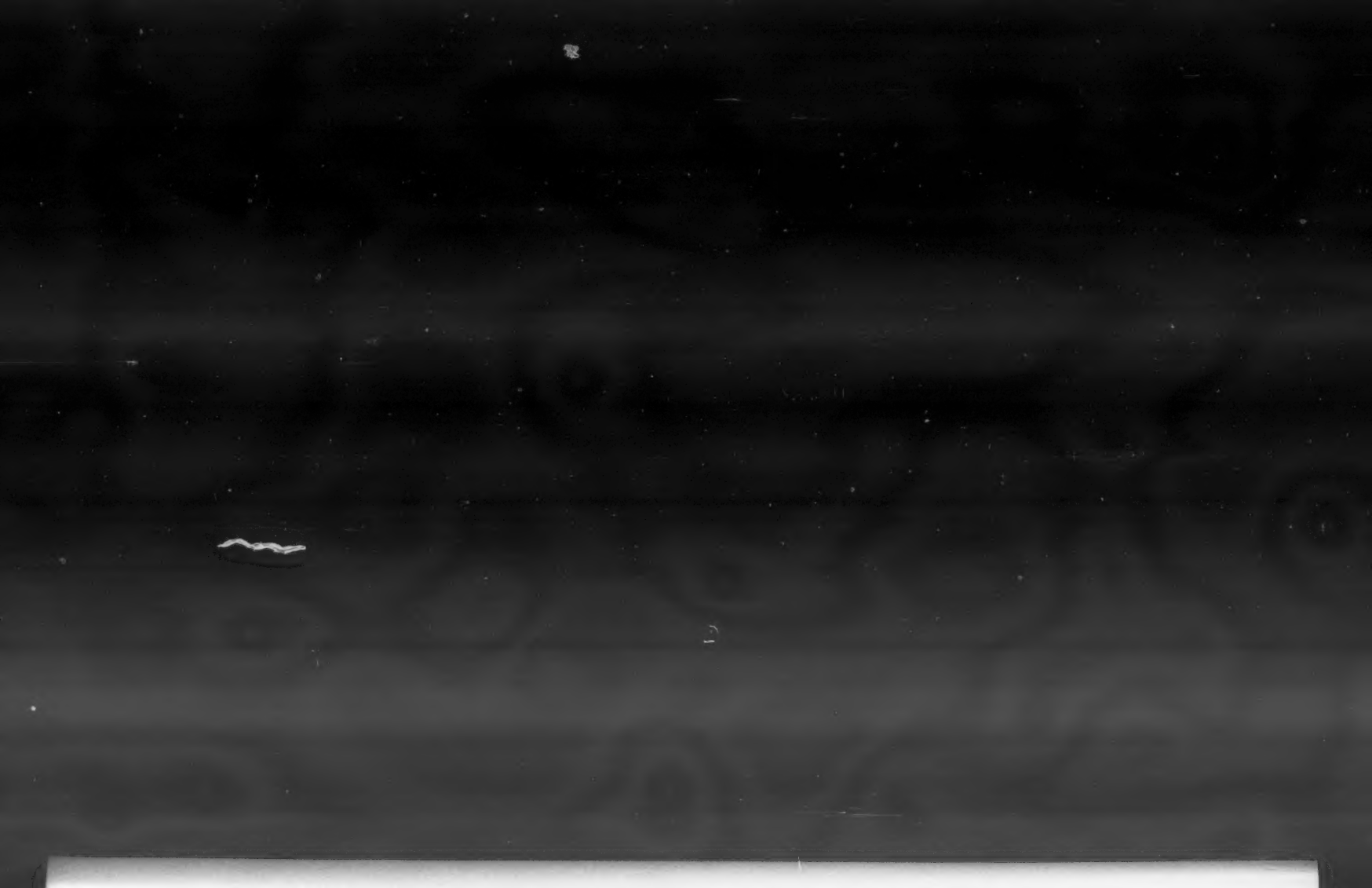
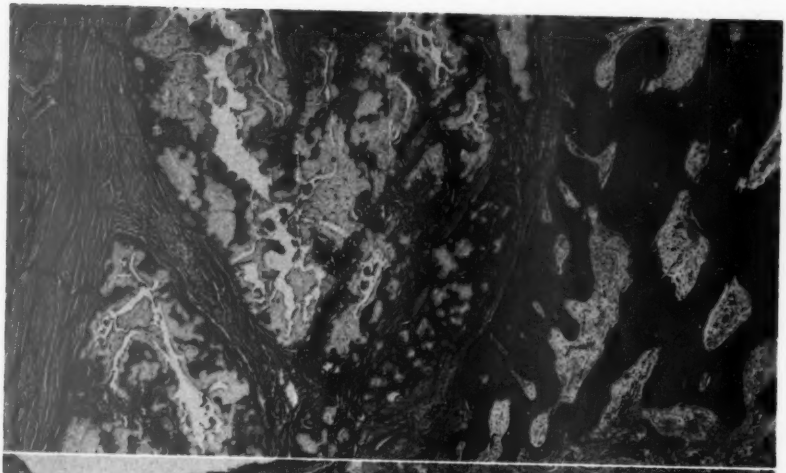
FIG. 18. Photomicrograph showing the general appearance of urate deposits within bone. × 55.

FIG. 19. Conspicuous calcification of urate deposits within the synovial lining and sublining connective tissue of a knee joint. × 55.

FIG. 20. From an articular surface (patella), showing loss of the articular cartilage (toward the left of the illustration) and replacement by urate-containing, vascularized connective tissue. × 180.





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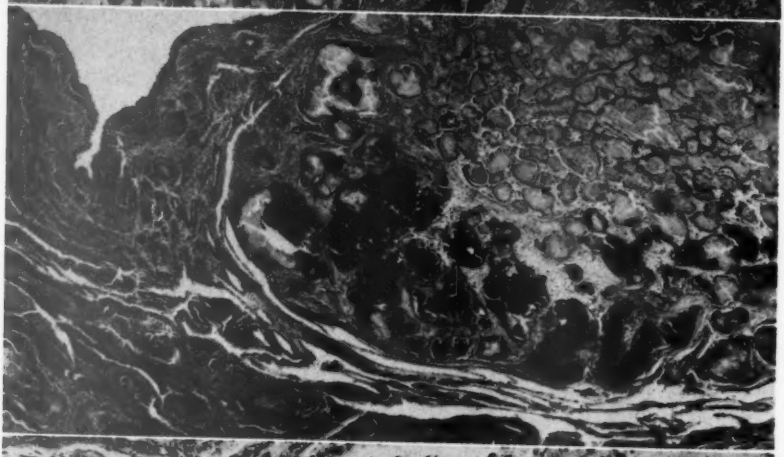

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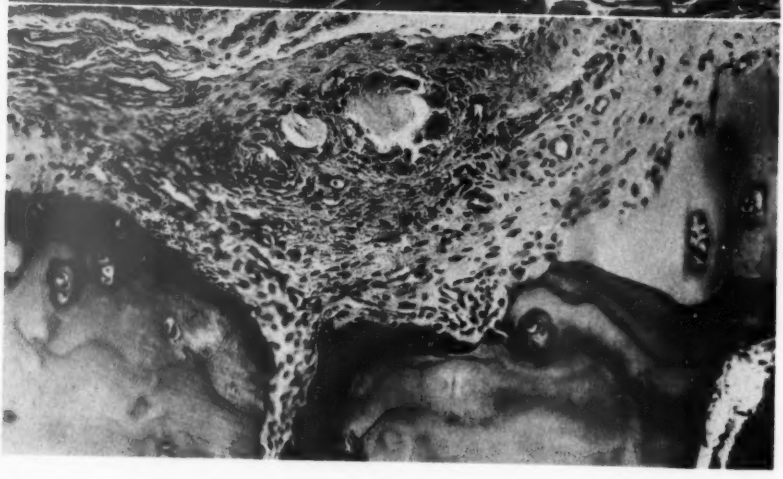

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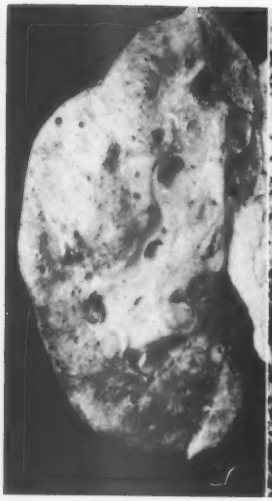
FIG. 21. Cut surface of the pale, scarred, and contracted kidney. Tophi were recognized within the medulla in the fresh state, but these are no longer clearly discernible in the preserved specimen.

FIG. 22. Urate deposition within a renal pyramid, in proximity to the pelvic mucosa. × 110.

FIG. 23. Crystalline deposits resembling urates within distended acini of the prostate. There is a foreign body reaction about them. The associated blackish deposits in the print represent calcium. × 125.



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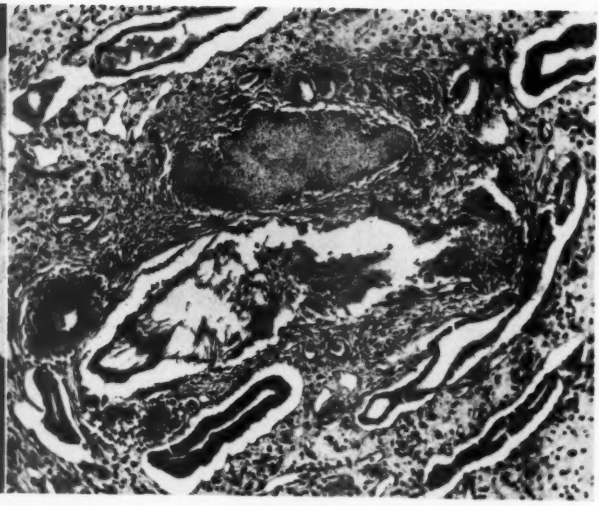

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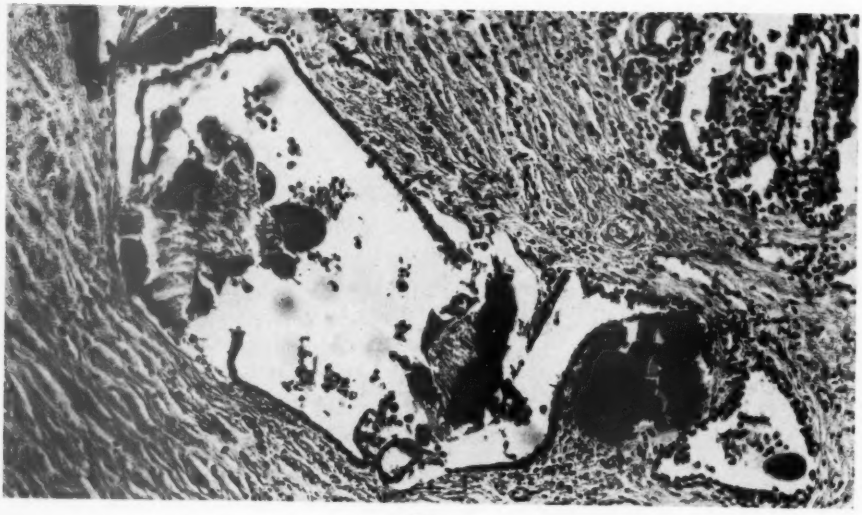

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THE APPLICATION OF AN INDUCED BRONCHIAL COLLATERAL CIRCULATION TO THE CORONARY ARTERIES BY CARDIOPNEUMONOPEXY

II. Hemodynamics and the Measurement of Collateral Flow to the Myocardium*

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The anatomical demonstration of channels that connect two vascular beds provides in itself no information regarding the volume, nor even the direction, of flow. The necessary functional determination of these modalities must be made without altering the pressure relationships, especially in a complex system involving several series of vessels. Preferably the animal should be intact, since the inevitable momentary physiologic variations in cardiac, vasomotor, and respiratory cycles may affect these pressure relationships in a way that cannot be duplicated by artificial pumps operating at constant pressures and outputs.

In approaching the problem of estimating the collateral blood flow to the heart after ligature of the left pulmonary artery and cardiopneumonopexy, the hemodynamics were investigated by catheterization and angiography, with the animal intact except for the required anesthesia and catheterization. The anatomical observations in these same animals have been described.[1] The angiography was performed in order to obtain a visual concept of the direction and rate of transfer of blood from the aorta into the collateral and other branches. It was hoped that the contrast medium might actually be traced from the collateral into the coronary vessels.

Moreover, it seemed that if a substance were introduced into the collateral vessels at a known concentration (Y), and if the concentration (Z) of this substance in the coronary sinus blood could be determined, then the collateral blood flow (CBF) could be calculated as a percentage of the total blood flow (TBF) reaching the coronary sinus, from the formula $CBF\% = Z/Y\ (100)$. Obviously the concentration Z would have to be determined before any of the injected

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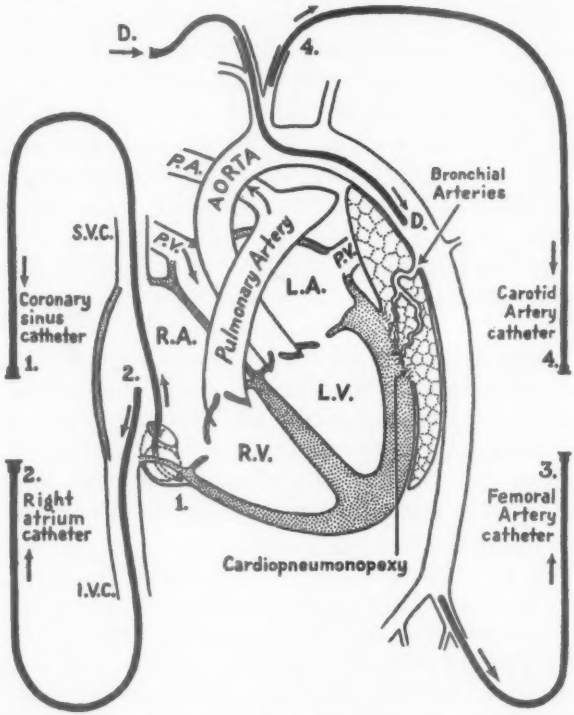
† James Hudson Brown Memorial Junior Fellow, 1954-55.

substance reached the ostia of the coronary arteries. Since TBF/min. can be determined by the nitrous oxide method[2-5] as applied by Bing and his associates,[6,7] CBF could be stated in ml./min. by applying the percentage obtained from the formula to TBF.

Methods

Placement and Functions of Catheters

With the dogs under sodium pentobarbital anesthesia (30 mg. per kg.), catheters were placed in appropriate positions under fluoroscopic guidance as indicated in Text-figure 1. Polyethylene catheters were



Text-figure 1. Cardiopneumonopexy after ligature of the pulmonary artery. The stump of the ligated left pulmonary artery is indicated. Bronchial arteries are shown traversing the left lung (lobular mosaic) directly to the heart where they anastomose with branches of the coronary arteries across the adhesions at the cardiopneumonopexy. The positions of the catheters as employed for determining collateral blood flow to the myocardium are indicated. The tip of catheter D is shown within the aorta just above the ostium of a major collateral system. Catheter 1 is shown in the coronary sinus with the balloon inflated proximal to its end, in order to prevent reflux from the right atrium. In practice, distention of this balloon is maintained only during the 30 second sampling interval. Catheter 3 is introduced via the femoral artery, but its tip is usually somewhat higher within the distal aorta.

made radio-opaque either by filling with 70 per cent Urokon, or by inserting short segments of brass tubing into the distal end. The end of catheter D was introduced via a carotid artery and its tip was placed beyond the aortic arch opposite the fourth rib, or slightly higher. It was thus at a distance from the coronary ostia, and yet well above the orifices of the major bronchial arterial collateral vessels as had been determined from the anatomical observations. The catheter that was introduced into the coronary sinus[8,9] (Text-fig. 1, no. 1) was provided with a double lumen so that a balloon proximal to the tip could be inflated during the period of withdrawal of blood from the sinus, in order to prevent reflux of blood from the right atrium. Evidence that the catheter had been properly placed* consisted not only of observing, with the aid of x-rays, the characteristic curve that it assumes within the sinus, but also of demonstrating that the blood withdrawn from this catheter was definitely of a darker color than the mixed venous blood obtained from the right atrium. Moreover, with the catheter in the sinus and the balloon inflated, the pressure could be shown to rise very rapidly. Further confirmation at the end of the experiment was obtained in some instances by performing a coronary sinus venogram,[10] i.e., introducing radio-opaque contrast medium into the coronary sinus retrogradely while the balloon was inflated, and by permitting the catheter to remain *in situ* until its position could be determined by direct inspection at necropsy.

The function of the catheter in the right atrium (no. 2, Text-fig. 1) was not only to provide blood for visual comparison with that from the coronary sinus, but also to permit the construction of a dye concentration curve that provided data regarding direct systemic flow from the aorta to the right atrium.

Catheter 3, tapping the femoral artery or abdominal aorta, was used to gain an estimate of the peak concentration of dye within the aorta distal to catheter D, and, therefore, presumably also within all of the distal branches of the aorta including the collateral vessels. This concentration would correspond to the datum Y in the formula given previously.

The function of controlling the possible reflux of dye against the aortic stream proximal to catheter D was subserved by catheter 4 in the second of the carotid arteries. Blood obtained from this catheter also provided knowledge of the "recirculation time," that is, of the time required for dye introduced via catheter D to pass to the systemic

* The privilege accorded the senior author of learning this technique under the personal guidance of Dr. Richard J. Bing at the University of Alabama is gratefully acknowledged.

arterial and capillary distribution of the distal aorta, systemic veins, right atrium, right pulmonary artery, pulmonary capillaries, pulmonary veins, left heart, and aorta to catheter 4. This recirculation time was considered the same as the time when blood in the proximal coronary arteries would first have become contaminated with the dye introduced via catheter D. In practice, catheter 4 was connected with the carotid artery by means of a T-tube which provided a bypass for the carotid blood until the time of the actual blood sampling. Since one carotid artery had already been interrupted for placing catheter D, it was considered wise not to interrupt the opposite carotid artery.

The Dye Concentration Curves

On the basis of accumulated experiences in similar applications, 0.5 per cent Evans blue (T-1824) was the dye of choice.[11] In actual performance catheter D was kept filled with dilute heparin in saline solution until the dye was introduced. Two ml. of dye, plus an amount (usually 1 ml.) sufficient to fill the dead space in the catheter, was introduced from a marked syringe on signal, as rapidly as possible, at which time blood for the simultaneous dye concentration curves was obtained from each of the other four catheters. The sampling was continued over a 30 second interval. Blood from the coronary sinus (catheter 1) and right atrium (catheter 2) was gently aspirated by syringe at a constant rate into polyethylene tubes (3.5 mm. internal diameter), each approximately 350 cm. long and marked in segments corresponding to 0.5 ml. With the balloon at the end of the sinus catheter inflated, it usually was found possible to obtain approximately 12 ml. during the 30 second interval. The blood column in the polyethylene tube attached to the right atrial catheter (no. 2) was advanced simultaneously and made to follow that in the sinus tube. The withdrawal was therefore at the same rate, and the final total volume was the same in both tubes. At the end of the withdrawal period the polyethylene tubes were clamped simultaneously at each of the segmental marks. Each segment then is representative of a fractional 0.5 ml. sample that corresponds to a definite time during the withdrawal period. These samples were harvested for analysis simply by cutting the polyethylene at each mark, introducing the blood into a test tube, centrifuging, and withdrawing the plasma with its content of dye for analysis. The analysis was accomplished in a Coleman Junior Spectrophotometer.

The blood from the femoral (no. 3) and carotid (no. 4) catheters

was permitted to flow under arterial pressure into test tubes carried on each of two rotating drums, by the method of Hamilton *et al.*[11] Each tube then contained a sample representative of the dye concentration in the respective vessels at a definite moment. This procedure was carried out beginning at the same signal, and over the same 30 second interval, as the sampling from catheters 1 and 2. After determinations of the dye concentrations, it was therefore possible to construct four simultaneous time-concentration curves for comparison.

It was found possible to repeat the dye study in most animals in 4 to 7 days, since the clots in the vessels that had previously been used for cannulation could be dislodged without difficulty.

Angiography

Bronchial arterial angiography was performed by introducing 70 per cent Urokon by a polyethylene catheter, the tip of which was in the same position as that of catheter D in Text-figure 1. Twenty to 25 ml. were introduced as rapidly as possible (usually within approximately 5 seconds) by means of a 50 ml. syringe compressed in a mechanical lever arrangement. During the injection the abdominal aorta below the renal arteries was momentarily compressed either by means of a loop of strong string or by manual pressure. This method proved more simple and reliable in our hands than cannulation of the fifth intercostal artery and retrograde injection of a contrast medium as practised by Nordenström.[12] One reason for the greater simplicity of the aortography as carried out in the present experiments is the variation in origin of the principal collaterals as determined in the casts. Urokon injected rapidly in such large volumes into the thoracic aorta resulted in intense spasm of the musculature, or in convulsions. Since the animals were sacrificed shortly after this procedure, its effect on survival cannot be reported.

In a few animals the coronary arterial system was injected directly, in one instance by accidental proximal malposition of catheter D, and in the other by intentionally introducing the catheter into the ascending aorta just above the sinuses of Valsalva, as has been shown to be successful for this purpose by others.[13-16] The carotid by-pass was clamped during this procedure, in order to prevent too rapid an injection of the Urokon into the cerebral vessels.

After the other angiographic procedures, the coronary sinus balloon was again inflated and approximately 5 ml. of the contrast medium was injected rapidly into the sinus, against the blood current,

while several radiographic exposures at 0.7 second intervals were made. This method was used to demonstrate the competency of closure by the balloon, as well as the correct position of the catheter tip within the sinus. Coronary venography has been described by Tori.[10]

Further Procedures

After angiography the animals were sacrificed by an overdose of sodium pentobarbital and an excess of heparin solution was introduced intravenously to keep the blood fluid. The catheters were left *in situ* so that their position could be ascertained at necropsy, which was begun within ½ hour. This procedure and the preparation of casts of the blood vessels of the heart and lung have been described.[1]

Observations

Angiographic Studies

Although angiography actually was the last of the experimental procedures, the results of these studies will be discussed first since they offer a graphic demonstration of the time factors involved in the collateral circulation. There was a close correspondence of the vessels as seen in the film and in the vinylite casts (Figs. 1 and 2).

Thirteen aortic angiographic studies were performed in 10 dogs. The first 5 may be considered developmental. Subsequently 8 satisfactory serial angiograms were made in 5 of the animals. In 4 of these dogs, collateral blood flow studies were performed also. It was therefore possible to obtain a correlation of the visual and angiographic data with the dye curves in various parts of the circulation. The film first showing dye escaping from the end of the catheter in the aorta was considered as representing 0 time. The subsequent films were made at 0.7 second intervals. The temporal sequence of various events is indicated in Table I, and illustrated in Figures 3, 4, 5, and 6.

Filling of the proximal bronchial arteries occurred within 0.7 seconds after the contrast medium appeared in the aorta at the level of origin of these vessels. Even when Urokon was introduced at the aortic valve cusps, proximal bronchial arterial filling occurred within 1.4 seconds. Within the next 0.7 seconds filling of distal bronchial arteries took place. In three instances "negative bronchograms" were demonstrated, one as early as the 0.7 second film, but always within 2.1 seconds of the dye injection. The term negative bronchogram has been coined by Nordenström[12] to designate the opacification in an angiogram of the walls of a bronchus richly supplied with bronchial arteries. Contrary to the appearance in the usual bronchogram, the lumen of the bronchus is radiolucent in sharp contrast with the opaci-

fied wall (Figs. 7 and 8). Fading of sharp outlines of the opacified bronchial arteries, with apparent diffusion of the medium into capillaries, usually took place in 3.5 to 4.2 seconds. Obviously this moment depends in part upon the duration of the injection of the contrast medium, and the values, therefore, are maximal for the time of transit through this segment of the circulation.

TABLE I

Analysis of Angiograms

Dog no.	Group	Interval, cardio-pneumono-pexy to sacrifice	Type of angiogram	Opacification time in seconds						Figure no.
				Coronary arteries	Bronchial arteries (proximal)	Bronchial arteries (peripheral)	Azygos	Pulmonary arteries	Pulmonary veins	
		max.								
335	B	7	Descending thoracic (lateral)		0.0	0.7	0.7	2.1	4.2	3
			Descending thoracic (anteroposterior)		0.7	0.7	0.7	4.2		4
353	C	6	Descending thoracic (lateral)		0.7	1.4				
320	D_1	12¼	Proximal thoracic	0.7	1.4	2.1		2.8		5
324	D_1	11½	Descending thoracic (lateral)		0.7	1.4		2.8		1, 7
			Proximal thoracic	0.7	1.4	2.1				
321	D_2	11¾	Descending thoracic		0.7	0.7		3.5		6

Evidence was sought for the opacification of the coronary circulation from the bronchial vessels or the reverse, but none was found. This may be the consequence of dilution of blood containing radio-opaque material with blood containing none, beyond the contrast necessary for radiographic visualization of the vessels. In one animal, dog 321, however, the radio-opaque braided wire that had been used for ligating the anterior descending branch of the left coronary artery served as a visible point of reference. In the lateral thoracic angiogram there was seen in a series of sequential films a vessel from the bronchial arterial plexus that appeared to terminate directly at the ligature. The coronary artery beyond the ligature was not visible. This collateral vessel did not pursue the direction of the intercostal arteries which were visualized in several of the films (Fig. 6). The termination upon the shadow of the suture, however, appeared quite constant, despite the obvious movement of the heart as demonstrated in the course of this serial angiography.

Opacification of the pulmonary artery could be seen in its initial

phases at between 2.1 and 4.2 seconds. In one instance (dog 320), it was apparent that the filling began from the distal branches of the pulmonary artery. This is to be expected from the studies of the casts, since the pulmonary artery is in fact a sac that ends blindly at the proximal end where it was ligated and has many points of connection with the bronchial arteries in its peripheral branches, whereby it is filled with blood. The opacification of the pulmonary artery persisted for at least 7 seconds and in one instance these vessels were still sharply outlined at 8.4 seconds.

Only in one instance (dog 335) were the pulmonary veins discerned. They became opacified at 4.2 seconds, 2.1 seconds after the pulmonary arteries. Both sets of vessels, together with the bronchial arteries, were visible in the same film, although the last mentioned were then beginning to fade (Fig. 3).

The very rapid opacification of the azygos vein, evident within 0.7 seconds in the case of dog 335 (Figs. 3 and 4), is of interest. This could be studied both in lateral and anteroposterior films. In a detailed analysis the pathway appears to be in large part from the aorta, to the intercostal arteries, capillary bed, and intercostal veins, which are successively opacified (Fig. 4). It is clear that the blood does not remain long in the systemic capillaries. This short circuit correlates well with the very rapid appearance of Evans blue dye injected at the same point as the contrast medium, and warns of the necessity of temporarily isolating the coronary sinus from the right atrium by the balloon method.

Analysis of Dye Concentration Curves

When the dye concentration curves in various parts of the circulation are compared, several differences between the control and experimental groups become evident. These are brought out in a representative curve for each group (Text-figs. 2 and 3), and in a tabulation of critical time periods from observations upon all of the animals (Table II).

In the experimental group the dye very rapidly reached a significant concentration (in excess of 0.1 mg. per cent) in the coronary sinus after having been introduced into the aorta (Text-fig. 2, curve 1). The level rose to 0.1 mg. per cent in an average of 2.7 seconds (range 1.0 to 4.5 seconds), while in the control group this level was not attained until 14.9 seconds (range 10 to 22.0 seconds), had passed. The 0.2 mg. per cent level was reached in 5.3 seconds (range 1.5 to 10.3 seconds) in the experimental animals, and in an average

of 19.0 seconds (range 13.5 to 22.8 seconds) in the controls. The sharpness of this difference is brought out by the fact that there is no overlap in any of the data for the respective groups. Repeated observations on the same animals also are remarkably close.

TABLE II

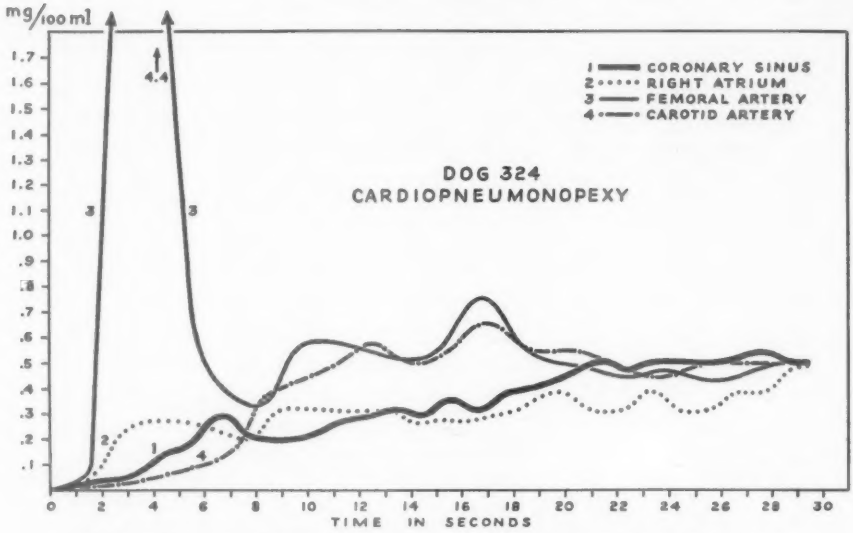
Analysis of Dye Concentration Curves

Cardiopneumonopexy

Dog no.	Group	Time in seconds						Lowest femoral artery level before recirculation (mg. %)
		To first femoral artery peak	Recirculation start	Recirculation peak	Recirculation peak to equilibrium	Sinus > 0.1 mg. %	Sinus > 0.2 mg. %	
353	C	10	13	14	6	3.0	8.0	0.62
355	C	4	10	14	12	1.0	2.0	0.20
		4	13	15	11	2.5	3.0	0.29
320	D_1	4	9	15	9	1.5	2.0	0.28
		3	10	15	7	1.3	1.5	0.30
324	D_1	5	10	18	6	4.0	10.3	0.20
		4	8	17	5	4.0	5.8	0.33
321	D_2	3	10	14	5	4.5	9.5	0.30
	Mean	4.6	10.4	15.2	7.6	2.7	5.3	0.32
				Control				
401		6.0	13	17	11	12.5	18.0	0.15
		6.0	13	18	12	10.0	13.5	0.20
402		8.5	14	17	13	16.0	22.8	0.20
		6.0	13	18	9	12.0	13.5	0.15
403		9.0	22	27	3	22.0	27.0	0.15
284		4.5	10	14.5	14	20.5	20.8	0.00
405		3.0	9	12	16	10.0	15.5	0.05
		5.0	12	15	9	16.5	20.0	0.07
	Mean	6.0	13.2	17.3	10.9	14.9	19.0	0.12

The rapidity of transit of the dye through the heart was remarkable, but the transit through the extracardiac systemic capillary bed (aorta to right atrium) was even more rapid. This was true in animals both of the control and experimental groups. This is exemplified in a control animal (Text-fig. 3, curve 2), in which the dye had reached a concentration of 0.1 mg. per cent in the right atrium 3.25 seconds

after injection into the aorta, and a concentration of 0.36 mg. per cent at 5 seconds. This early peak was close in timing to that of the initial peak of dye concentration in the femoral artery, an observation confirmed in all other curves in which the data for the right atrium were available. In the control animals the right atrial peak

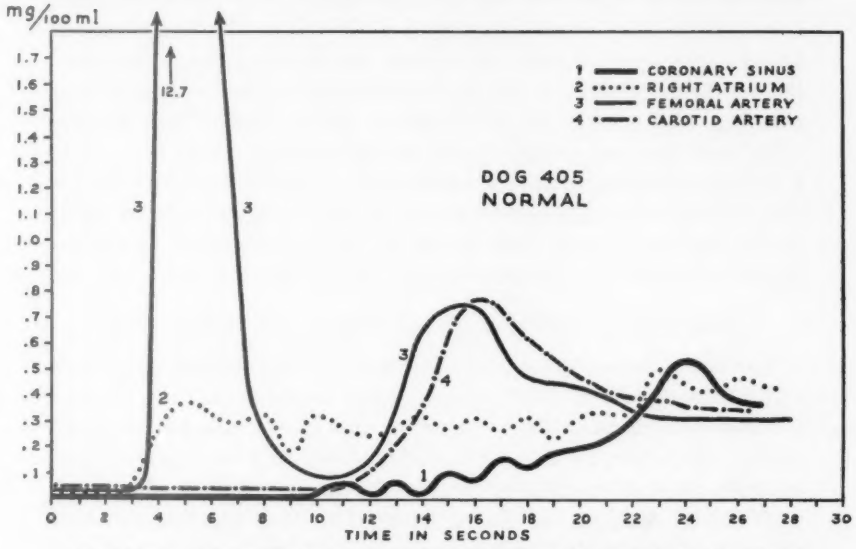


Text-figure 2. Representative dye concentration curves as obtained from the 4 catheters positioned as in Text-figure 1, from an animal with a cardiopneumonopexy. The Evans blue concentration has risen to 0.2 mg. per cent at 5.8 seconds, before the beginning of significant recirculation into the proximal aorta and coronary ostia. The initial peak dye concentration attained in the aorta in this instance was 4.4 mg. per cent as indicated by an arrow in the diagram. An angiogram and a cast from this dog are illustrated in Figure 1. The correct position of catheter 1 in the coronary sinus and the competency of its balloon were demonstrated by angiography at the conclusion of this experiment (Figs. 9 and 10).

preceded the appearance of dye in the coronary sinus by an average of over 8 seconds, while in the experimental group the dye in the sinus appeared at a time very close to that of the appearance of dye in the right atrium. In the experimental group the coronary sinus curve crossed the 0.1 mg. per cent level at 2.7 seconds, an average of 7.6 seconds *before* the dye could have reached the ostia of the coronary arteries (beginning of "recirculation"), while in the control group the 0.1 mg. per cent level was not attained in the coronary sinus until 14.9 seconds on the average and 1.7 seconds *after* the beginning of recirculation (13.2 seconds).

The dye concentration curves in the femoral and carotid arteries (Text-figs. 2 and 3, curves 3 and 4) provide an estimate of the time

when "recirculation" begins, i.e., when the dye, after injection into the thoracic aorta, makes its appearance in the aorta proximal to the point of injection, having passed from the systemic capillaries through the right heart and vessels of the normal lung, to the left heart and ascending aorta. The rise of dye concentrations in the carotid and



Text-figure 3. Dye concentration curves from a representative control dog. In contrast with the experimental dog, the Evans blue concentration did not reach 0.2 mg. per cent until 20.0 seconds, clearly after the peak of the first recirculation. The initial peak of dye concentration was 12.7 mg. per cent (arrow). There is an early high peak in the atrial dye level, while the level in the sinus remained at zero. This again demonstrates competency of the balloon of the sinus catheter.

femoral arteries to the second peak was almost simultaneous, and reached approximately the same level (in Text-figs. 2 and 3, compare curves 3 and 4. Actually, the rise seemed to be somewhat earlier in the carotid artery, as might be expected from the shorter distance traversed by the blood in its course from the heart to reach this vessel.

There are certain notable general differences in the arterial dye concentration curves of the control and experimental animals that are exemplified in Text-figures 2 and 3. The recirculation began earlier in the experimental group (average 10.4 seconds as compared with 13.2 seconds in the controls), and the peak of the recirculation curve also was evident sooner in the experimental dogs (average 15.2 seconds in comparison with the controls, average 17.3 seconds). Further-

more, in the experimental group, the dye concentration in the distal aorta (femoral artery sample, curve 3) did not fall to as low a level before recirculation began as in the control group. The respective average levels for the experimental and control groups were 0.32 mg. per cent and 0.12 mg. per cent. Here the highest level in the control group equalled the lowest in the experimental. The third difference is reflected in the shapes of the curves after recirculation has occurred: In the control groups there were usually two sharp peaks as illustrated in Text-figure 3, while in the experimental group the curve was more complex, with a series of lower waves, the first being only slightly taller than the rest, as equilibrium was approached (Text-fig. 2). As a further reflection of this phenomenon, it appeared that equilibrium, i.e., the time when the concentration of dye is the same in all parts of the circulation, was attained sooner (at 22.6 seconds) in the experimental than in the control group (29.7 seconds).

Estimation of Collateral Blood Flow to the Myocardium

The two values necessary for calculating the collateral blood flow (CBF), as described under Methods, can be obtained from the dye concentration curves. Two alternative procedures can be employed in the calculation, to which the designations peak method and area method, respectively, will be applied in the text and in Table III.

The Peak Method. In the first procedure it is assumed (1) that the peak of the femoral artery curve (in Text-figs. 2 and 3, curve 3) represents the maximum concentration of dye per ml. of blood reaching the collateral circulation (concentration Y); (2) that the peak of the first coronary sinus curve before recirculation (Z) represents the least dilution of blood containing concentration Y, after it has become mixed with blood from the coronary ostia that contains no dye. Actually, since there is a tendency toward dulling the peak representing the concentration in the coronary sinus, for reasons considered later in the discussion, Z is a minimal value. It is therefore obvious from the formula $CBF\% = Z/Y \times 100$ that CBF calculated in this fashion represents a minimal value.

The Area Method. An alternative, and possibly more desirable, way for obtaining Y and Z is to determine the relative dye concentration in the aorta and coronary sinus over an interval of time. Ideally, the entire areas under the curves before recirculation takes place should be used. Some practical difficulties exist in the way of realizing this ideal, since artefacts, such as hemolysis, can cause some part of the apparent "dye level" below 0.1 mg. per cent, and since the moment of actual entry of dye, and moment of the beginning of

recirculation cannot be determined exactly. As a compromise, the 4 second intervals centered upon the first peaks of the aortic and sinus dye curves, respectively, were used as a basis of comparison.

TABLE III

Collateral Blood Flow to Myocardium

Dog no.	Group	Interval, cardio-pneumo-pexy to sacrifice	Date of flow study	Total flow into coronary sinus	Collateral flow into coronary sinus			Evidence for position of catheter			
					% of total						
					Peak method	Area method	Peak	Color of blood	Chest film	Sinus angio-gram	Necropsy
		mos.		*ml./min.**			*ml./min.*				
353	C	6	2/11/55		16.2	18.7		+	+		
			2/15/55		14.3	19.3		+	+		
355	C	6	2/18/55	83	(26.2)	(37.6)	(21.8)				
			2/22/55		10.9	13.7	9.5	+			+
320	D_1	12¼	2/25/55	58	9.5	13.9	5.5	+	+		
			3/1/55		9.0	12.8	5.2	+	+		+
324	D_1	11½	3/11/55	50	5.6	11.0	2.8	+	+		
			3/15/55		6.6	9.9	3.3	+	+	+	+
321	D_2	11¾	3/4/55	48				+	+		
			3/7/55		4.1	6.3	1.9	+	+		†
401	Con-trol		3/25/55	78	0.3	0.9	0.2	+	+		
					0.3	0.7	0.2	+	+		
402	Con-trol		4/1/55	55	0.6	1.4	0.3	+	+		
			4/5/55		0.17	0.5	0.09	+	+	+	†
403	Con-trol		4/8/55		0.1	0.17		+	+		
284	Con-trol		4/11/55	54	0.0	0.0	0.0	+	+		
405	Con-trol		4/18/55		0.6	0.2		+	+		
			4/25/55		0.3	0.06		+	+	+	

* ml./min./100 gm. of heart muscle.

† In animal 402 the catheter obviously became dislodged during the convulsions associated with aortography, or during subsequent transfer from the x-ray room, since retrograde sinus angiography had demonstrated it in position; the same may have been true for dog 321.

Data regarding dog 355 (2/18/55) are shown in parentheses to indicate lack of confidence, since the position of the coronary sinus catheter was not established with certainty (see text).

In practice the areas were compared by plotting the desired portions of the curves on graph paper on an enlarged scale, cutting out the areas enclosed by the appropriate ordinates and base line, and weighing the paper.

The data for total and collateral blood flow, as calculated by both

methods, are recorded in Table III. Since the *sine qua non* of validity is that catheter 1 be, in fact, sampling blood from the coronary sinus, the evidence for position of the catheter also has been recorded. The high values for collateral flow obtained in the first experiment with dog 355 (2/18/55) may be spurious since the position of the catheter was not established adequately in this instance. They will therefore be disregarded and are indicated in parentheses in Table III. The data obtained in duplicate experiments agree remarkably well for each of the three dogs (nos. 353, 320, and 324) when calculated by either the peak or area procedure. In these animals the experiments were repeated on separate days. This may be interpreted as an indication of the validity of these methods of approaching the problem. From what has been said regarding the "averaging" tendency of the polyethylene tube sampling method, it might be expected that percentages determined by the area method would be higher than those obtained by the peak method, and this is actually the case. The values for CBF by the peak method range from 4.1 to 16.2 per cent of total flow, and from 6.3 to 19.3 per cent of total flow by the area technique. The true flows probably lie somewhere between the results obtained by the two procedures. In the control animals a dye level of 0.1 mg. per cent was not reached in any instance before recirculation took place; nevertheless, lower values are used as if they were significant in the calculation of the recorded flows, none of which exceeds 0.6 per cent of total flow as obtained by the peak method, or 1.4 per cent of total flow by the area method.

Discussion

These observations demonstrate that with cardiopneumonopexy of a lung with a ligated pulmonary artery there is a measurable collateral inflow of blood to the myocardium. The data do not reveal the relative importance of the various types of collateral vessels that were observed in the anatomical investigation, but only the total inflow. Since the connections are of precapillary size and occur only with coronary arteries and never directly with the veins, the collateral blood flow must have the same capillary distribution as any blood reaching the distal coronary arteries.

The mechanisms that determine flow from the collateral vessels toward the heart, at least during a part of the cardiorespiratory cycle, are unknown. It may be that when the pressure pulses transmitted to a point of anastomosis from the proximal coronary arteries on the one side and from collateral vessels on the other are sufficiently out

of step, the latter pressure may at some phases exceed the former.

The accuracy of the quantitative estimations of the collateral blood flow may be questioned, since they are based on the assumption that the peak level in the collateral vessels does not differ significantly from that in the distal aorta. An indication that this assumption is not far removed from fact is the reproducibility of the results: the estimates by two methods of collateral flow performed in duplicate several days apart were remarkably close, even though the initial peak concentrations of dye in the distal aorta varied considerably.

It is of note that the larger collateral flows were observed in dogs, the coronaries of which had been wrapped with irritating polyethylene at their sources, even when there was no physical reduction in the lumina of these vessels. Further work is necessary to establish these isolated observations on a statistically significant basis.

Some fraction of any spectrophotometric reading corresponding to a level of less than 0.1 mg. per cent Evans blue may result from hemolysis in the sample. This is more likely to occur in material removed by aspiration, rather than by the rotating drum method. The spread of dye-containing blood among adjacent fractions from segmentation of the polyethylene may also be a factor, as will be discussed.

The method of sampling blood from the coronary sinus and right atrium is not ideal. Technical skill is necessary to aspirate the blood at the constant rate necessary to obtain a meaningful curve. Some dulling of the peaks and valleys of the dye concentration curve must inevitably occur, since there is doubtless some distortion from adherence of dye or blood to the tubing as the column of blood is aspirated, and also some diffusion during the process of clamping to isolate the segmental samples. Each point on a curve obtained by this technique, therefore, tends to approach the mean of concentrations over adjacent time intervals. Certain observations, nevertheless, speak for the usefulness of the method: (1) The demonstrated small peaks, as for example, the early low rise in the dye concentration in the coronary sinus and atrium; (2) the clear-cut differences between the curves for the control and the experimental groups; (3) the near identity of curves obtained by a simultaneous sampling by two separate polyethylene tubes. This last mentioned observation was made in what might be termed a "useful accident" when catheter 1 inadvertently slipped out of the coronary sinus, before the Evans blue was introduced into the aorta, in the second experiment upon control dog 284. The chest film made immediately afterwards demonstrated

the malposition of the catheter, as did the fact that a coronary sinus angiogram could not be obtained but showed that the radio-opaque material had diffused freely into the atrium.

It is surprising that the peak of the first recirculation curve in the aorta is always considerably higher than the dye concentration in the right atrium at any preceding time. It might be expected that the right atrial curve would reach a peak similar to the aortic recirculation peak but preceding it by an interval corresponding to the circulation time from the right atrium to the aorta. One possible explanation of this apparently anomalous situation is failure of adequate mixing in the atrium of two streams of blood containing disparate quantities of dye. The less perfect mixing in the atrium, as compared with that in the pulmonary artery, has been discussed by Warren, Stead, and Brannon.[17] In the present instance it is to be expected that, on the first circulation, most of the dye injected into the descending thoracic aorta would be brought to the heart by the inferior vena cava and that only the relatively small quantity arriving via the azygos system would be brought in by the superior vena cava. If inadequate mixing were the explanation, there should be occasions when the right atrial level actually exceeds the level of the aortic recirculation peak, but this was never observed in the present work. A better explanation probably lies in the sampling method, with the greater tendency of the curves obtained thereby to represent concentrations close to the mean.

The differences that were noted in the arterial dye concentration curves are probably based on the existence, in the experimental group with ligated pulmonary artery, of what is, in effect, a transpulmonary shunt between the right and left atria. This would explain the more rapid recirculation, the higher dye concentration before the onset of recirculation, and the less regular shape of the curve beyond the first peak, in the experimental animals as compared with the controls.

Summary and Conclusions

A bronchial collateral circulation induced in the lung by ligature of the pulmonary artery has been demonstrated to carry a considerable quantity of blood to the heart when implemented by cardiopneumonopexy. From the study of Evans blue concentration curves in the aorta and coronary sinus after injection of the dye into the aorta beyond the arch, but above the orifices of the major collaterals, the minimal collateral blood flow has been estimated in various animals to be from 4.1 to approximately 16 per cent of total inflow into

the coronary sinus, even in the absence of demonstrable myocardial ischemia. These functional data yield no information as to which of the systems of collaterals—transpleural or retrocardiac—serves as the major pathway.

The dye curves correlate well with angiographic observations in demonstrating the very rapid passage of blood from the aorta through the collateral system. In animals with cardiopneumonopexy, the Evans blue reaches levels in excess of 0.2 mg. per cent in the coronary sinus at approximately 5.3 seconds after injection of the dye into the aorta, definitely before there has been recirculation; while in the control animals this level is not obtained until an average interval of 19 seconds has passed, definitely after the dye has made the circuit to the proximal aorta and coronary ostia. By angiogram the circulation time from the aorta to the pulmonary veins of the lung with the ligated pulmonary artery was found to be 4.2 seconds.

There is at least one instance with angiographic evidence of the passage of the contrast medium from the bronchial collateral circulation to a coronary artery at a point of ligature.

It may be expected that, when the pressure in the vessels of the lung exceeds that in the coronary arteries, as in many instances of transposition of the great vessels, a relatively large flow would occur from the pulmonary arteries toward the heart after simple cardiopneumonopexy.

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LEGENDS FOR FIGURES

FIG. 1. Dog 324. Lateral view of cast for comparison with angiogram shown in Figure 2. The right lung has been removed, leaving only the stumps of its major bronchi and the carina of the trachea. Two major bronchial arteries, A and B, are seen leaving the aorta (Ao) to contribute to a dense plexus of vessels that proceeds anteriorly beneath the carina of the trachea to become distributed within the lung, and ultimately to contribute both retrocardiac and transpleural collaterals to the heart. The coronary arteries have been injected with plastic, and the cast has been retained in its relationship with the bronchovascular structures. Intercostal vessels arch posteriorly to the aorta before assuming a more lateral position.

FIG. 2. Dog 324. In the angiogram made just after the dye concentration curve illustrated in Text-figure 2, and just before the animal was sacrificed for preparation of the cast shown in Figure 1, the two major bronchial arteries, A and B, possess the same relations to each other and to the subcarinal plexus as demonstrated in the cast (Fig. 1). Within the vaguely defined shadow of the heart are shown catheter 1 within the coronary sinus and catheter 2 within the right atrium. The latter has in this instance been introduced via a femoral vein. This may be compared with other roentgenograms from this dog reproduced in Figures 7, 9, and 10.


A
B
Ao
1
D
A
B
2
1
2
Ao

FIG. 3. Dog 335. Lateral angiographic series. At 0 seconds the proximal bronchial arteries are already filled in the first film. At 0.7 seconds the intrapulmonary distribution of the bronchial arteries is opacified. The outline of the azygos vein is already evident, and can be identified by reference to the 2.1 second film (Az). At 2.1 seconds the ligated pulmonary artery is becoming opacified, but is shown more clearly in the 2.8 seconds film (P.A.). At 3.5 seconds the bronchial arteries, not as clearly seen in the previous films, are evident. At 4.2 seconds the pulmonary vein has become defined (compare with P.V. as marked at 4.9 seconds).

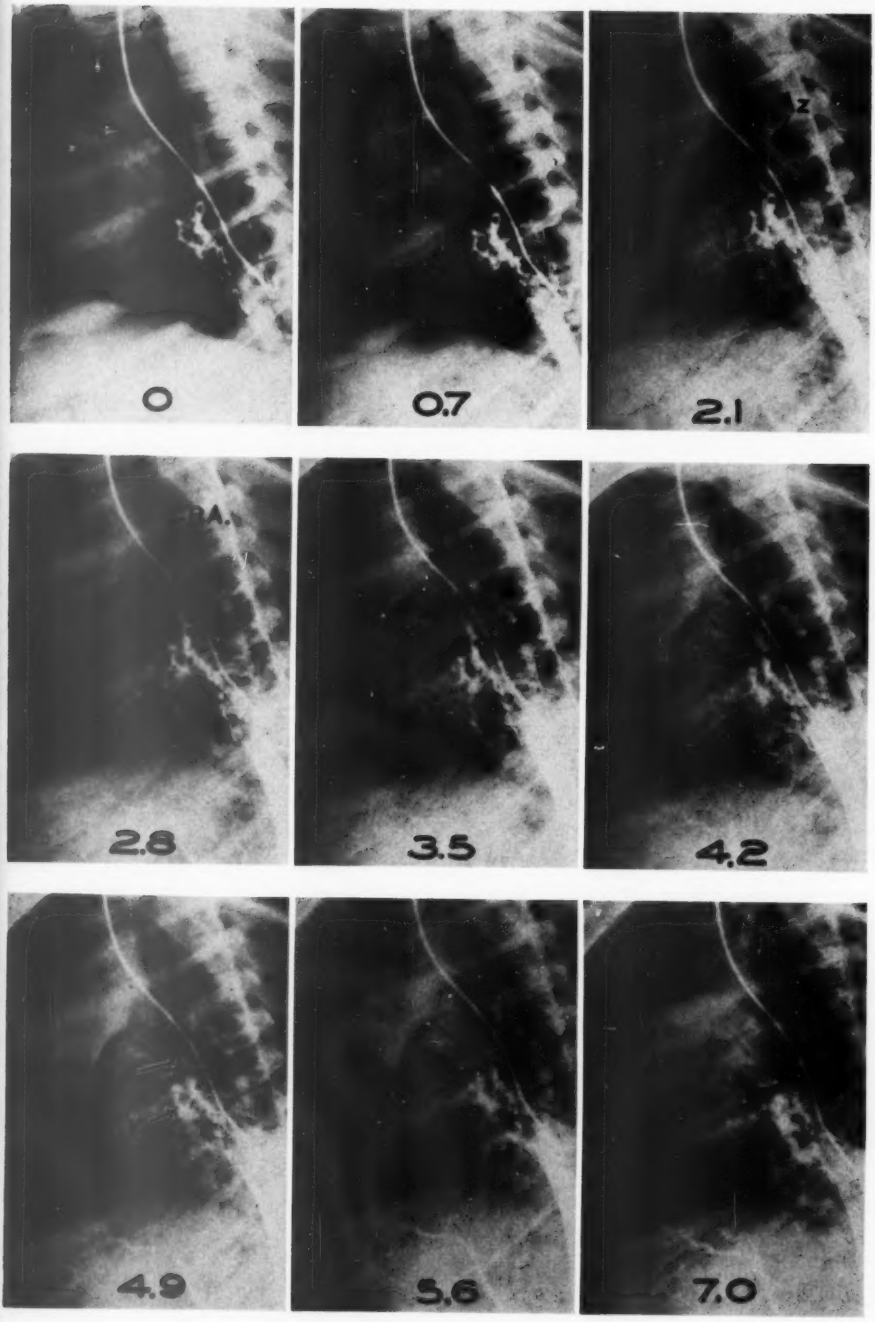
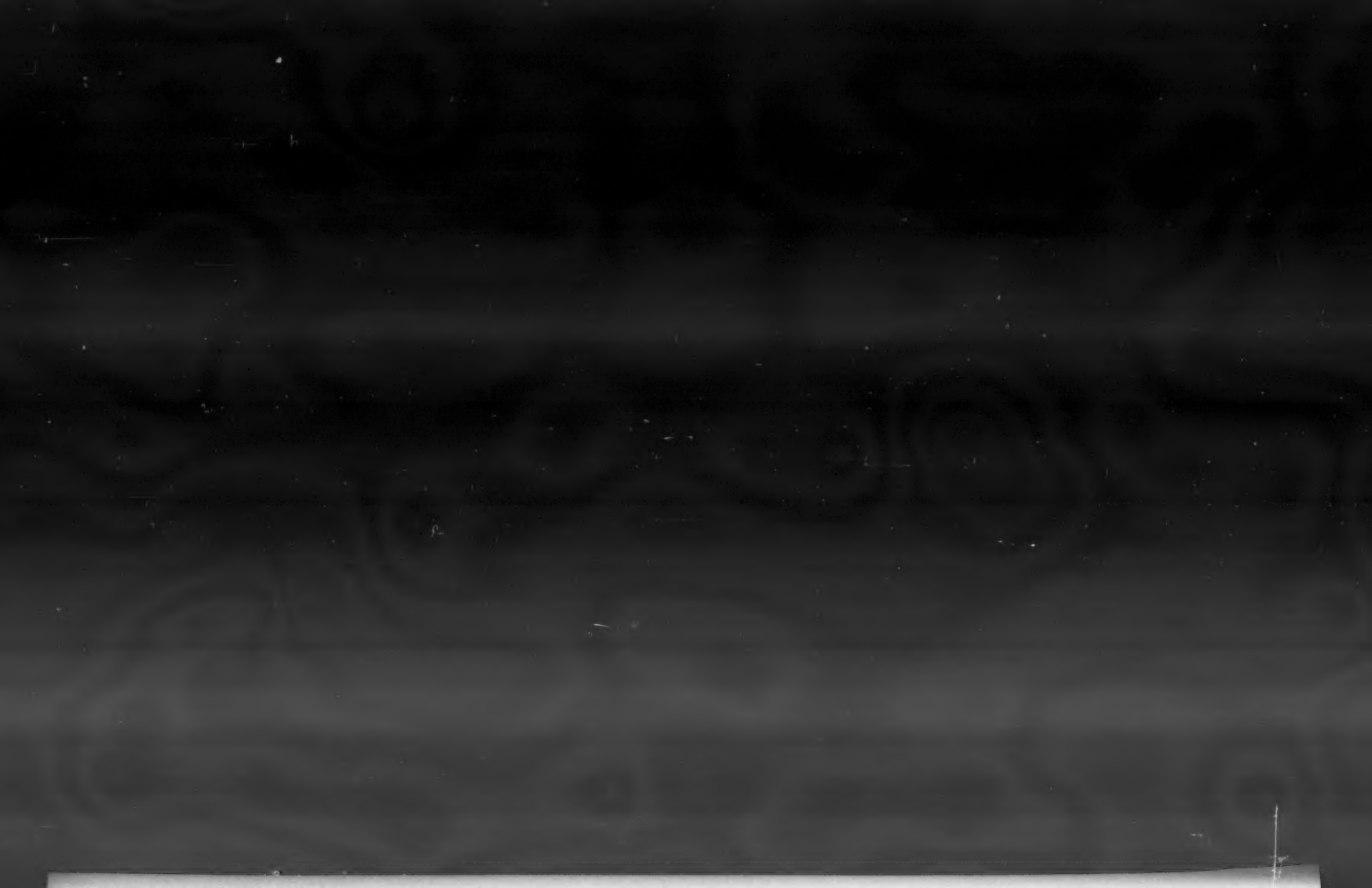

0
0.7
2.1
2.8
3.5
4.2
4.9
5.6
7.0

FIG. 4. Dog 335. Anteroposterior angiographic series. Time sequence is similar to that of Figure 3, but the pulmonary artery is not well opacified until 4.2 seconds. At 0.7 seconds an intercostal artery (I.A.) and an intercostal vein (I.V.) are shown together. There is also initial filling of the azygos vein (Az). This observation demonstrates a pathway by which the right atrium quickly receives blood from the aorta, as indicated also by the dye concentration curves of Text-figures 2 and 3.



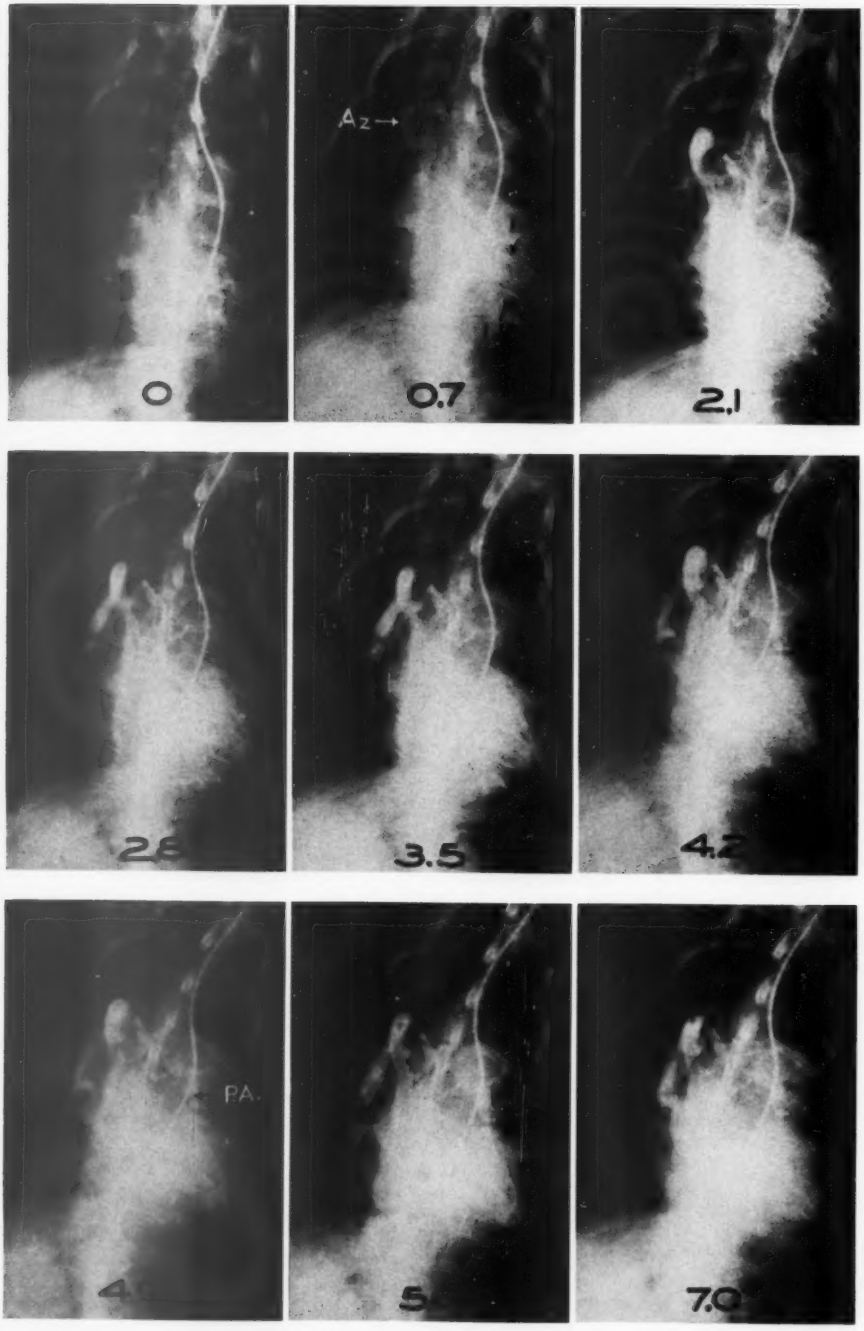


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FIG. 5. Dog 320. Proximal thoracic angiogram. Urokon injected retrogradely from a catheter in the brachiocephalic artery. At 0.7 seconds the first filling of the coronary arteries occurs. The outlines of the sinuses of Valsalva are well seen. At 1.4 seconds the coronary arteries are better shown. The right coronary artery (R.C.) appears to project anteriorly since the heart has been rotated to the left. The large left circumflex is demonstrated (L.C.). The aorta at the level of the ostia of the bronchial arteries has now become opacified, and the latter vessels have received some of the Urokon. There is no evidence that the bronchial arteries have received any of the contrast medium from the coronary vessels. At 3.5 seconds the pulmonary artery, which was initially opacified at 2.8 seconds, is more sharply outlined.

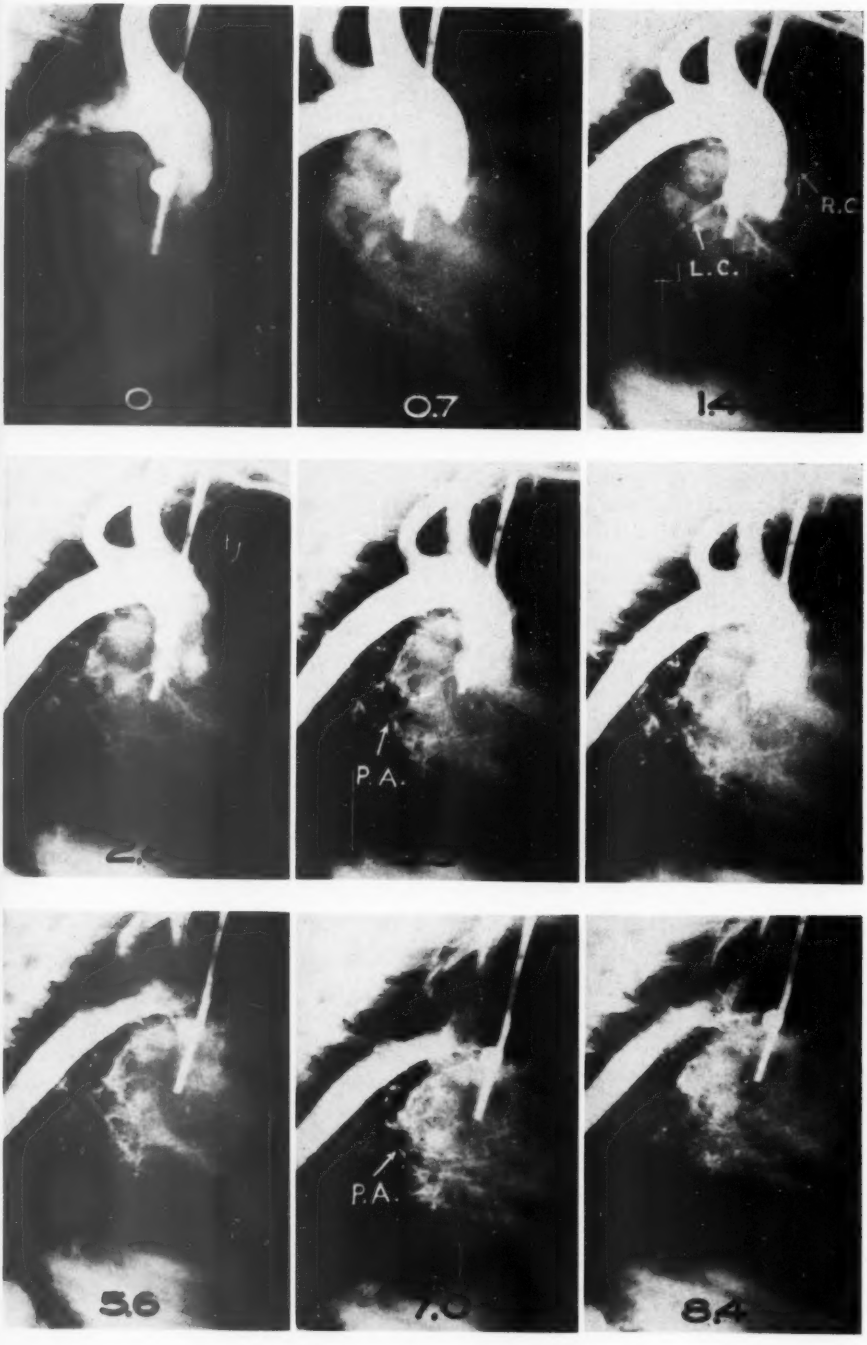
0
0.7
R.C
L.C.
1.4
P.A.
5
P.A.
5.6
7.0
8.4

FIG. 6. Dog 321. Demonstration of bronchial collateral to region of ligature of coronary artery. In this instance a braided wire had been used to ligate the anterior descending branch of the left coronary artery 3¾ weeks after interruption of the left pulmonary artery and cardiopneumonopexy. Animal sacrificed 11¾ months later. At 1.4 seconds an opacified vessel is seen to terminate at the wire. At 2.8 seconds it has been crossed by an opacified intercostal artery (I.A.) that is identified by its course parallel to others at various segmental levels. The collateral continues in an opacified state, and is still clearly seen at 4.2 seconds terminating upon the ligature, despite the movement of the beating heart. By this time most bronchial vessels appear blurred, and the pulmonary artery (P.A.) has become opacified. Catheter 1 is shown with its tip in the coronary sinus, and catheter 2 lies within the right atrium.

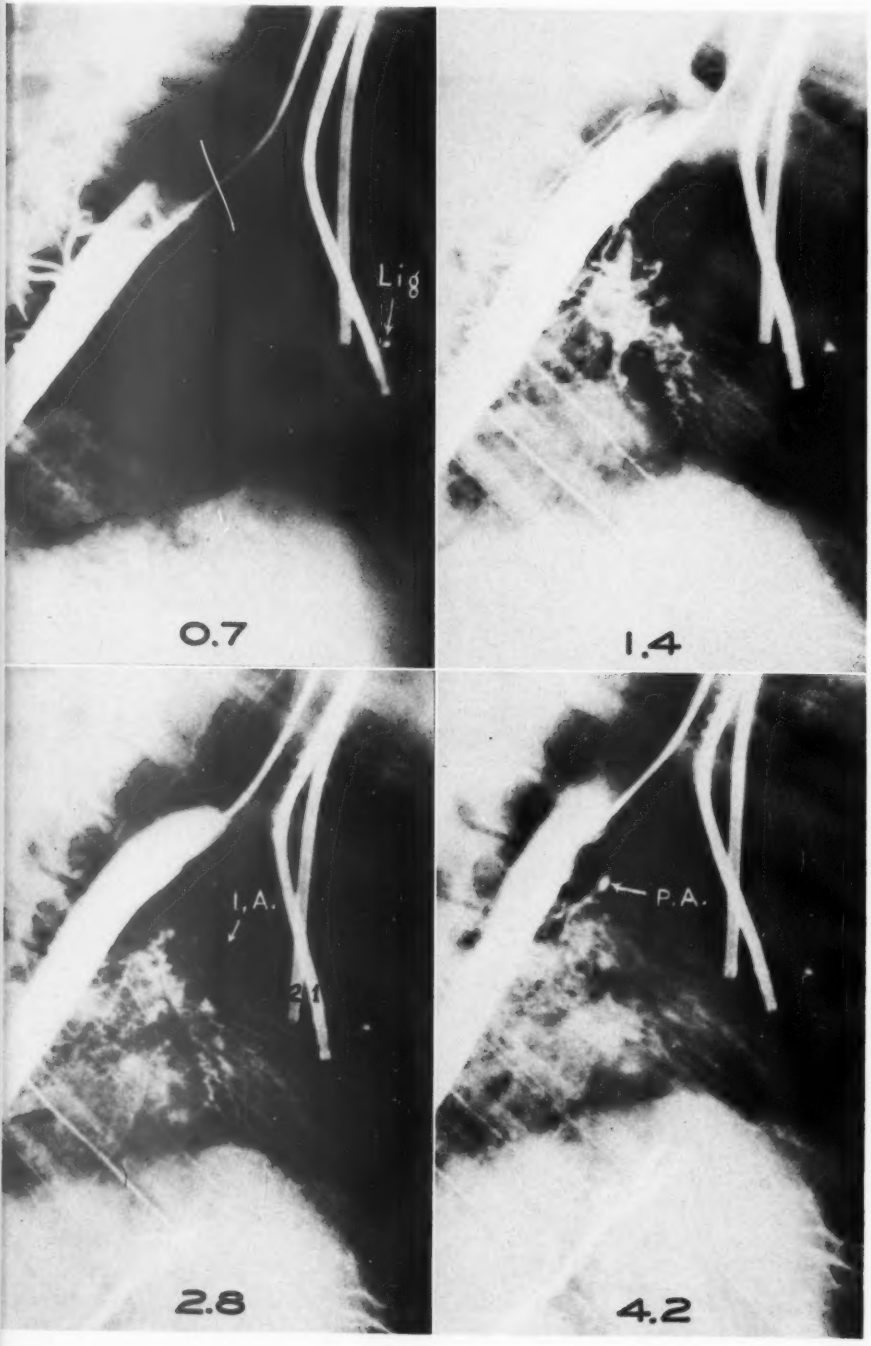

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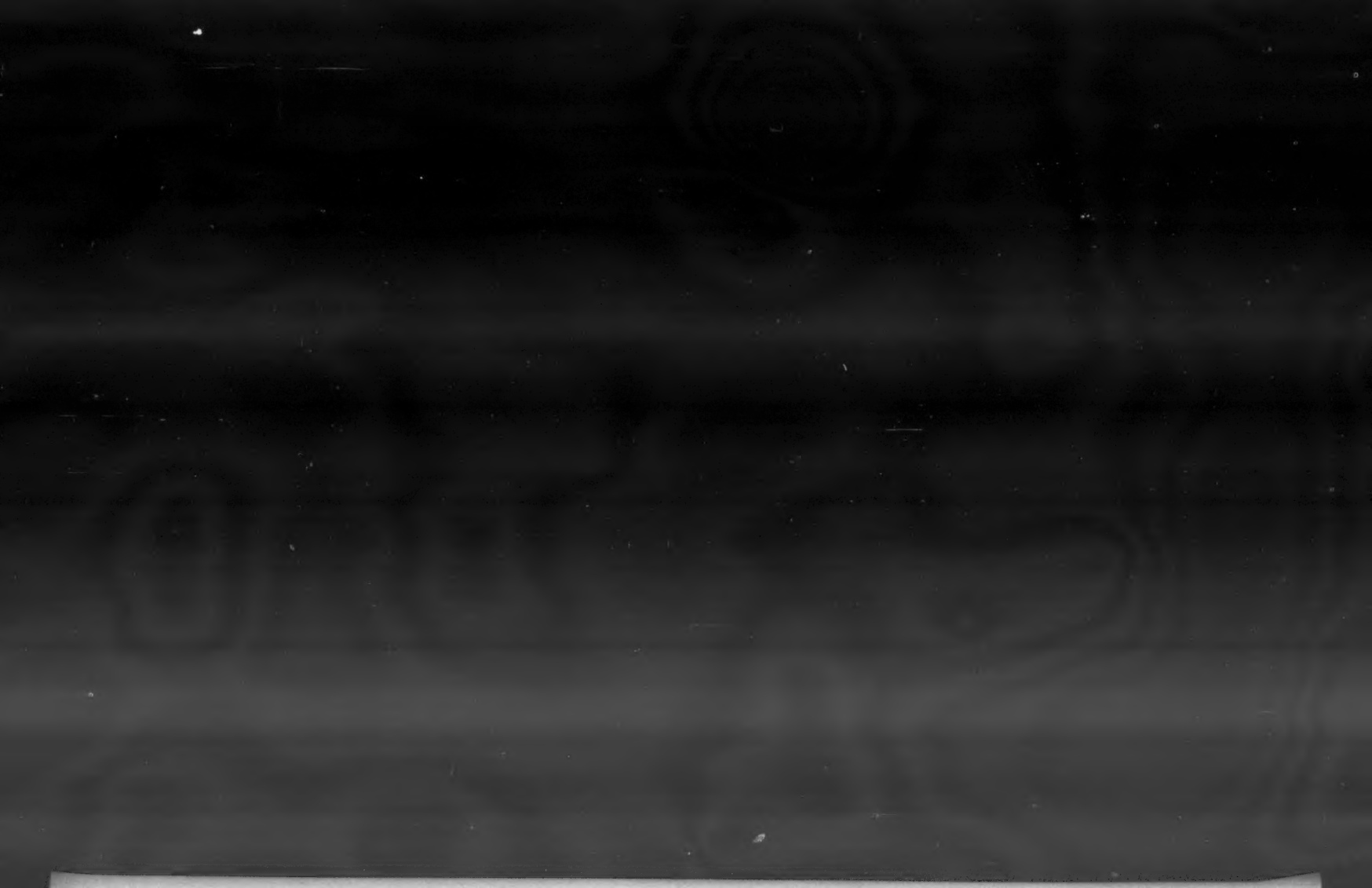
FIG. 7. Dog 324. "Negative bronchogram," at 2.1 seconds, lateral view. The left upper lobe bronchus is clearly outlined by the plexus of five bronchial arteries in its walls. Compare with Figure 1.

FIG. 8. Dog 350. "Negative bronchogram." The lower lobe bronchus is well shown. Anteroposterior view.

FIG. 9. Dog 324. Coronary venogram. The balloon has been inflated with Urokon, and this material is also being injected into the coronary sinus. The aortic injection catheter has been moved from its posterior position, shown in Figure 2, to the ascending aorta for a coronary arteriogram.

FIG. 10. Dog 324. 1.4 seconds after film illustrated in Figure 9. Further filling of the sinus and initial filling of some of its tributary veins are demonstrated here.



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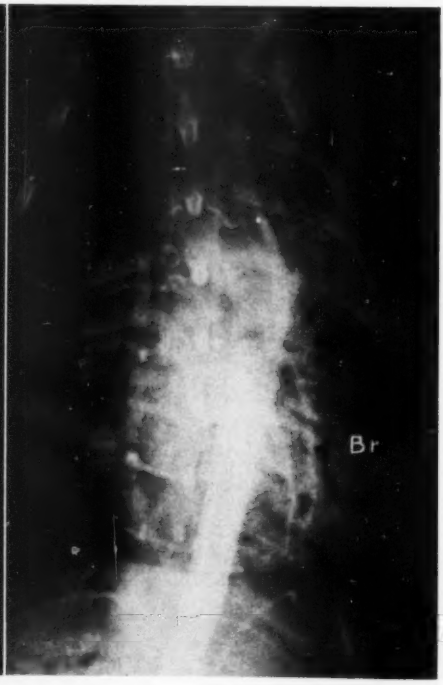

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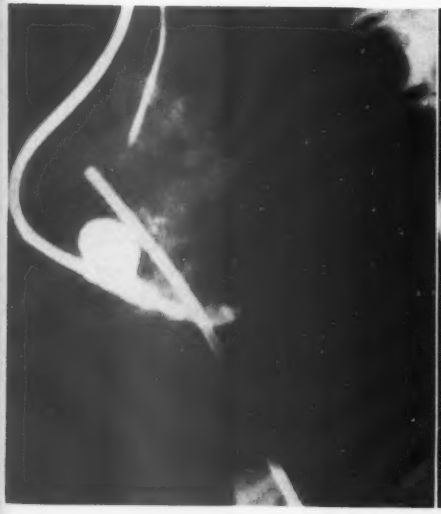

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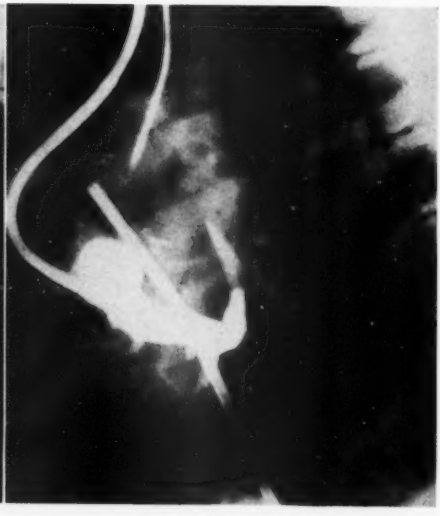

10

MULTIPLE SMALL ARTERIOVENOUS FISTULAE OF THE LUNGS*

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Large arteriovenous fistula of the lungs can no longer be considered in the category of rare lesions. Since the first correct clinical diagnosis was made by Smith and Horton[1] in 1939, well over 100 cases have been described and undoubtedly many others are unreported. Several excellent comprehensive reviews have been published, pointing out the relationship of these lesions to Rendu-Osler-Weber disease, or hereditary hemorrhagic telangiectasia.[2-6] Of the 149 reported cases of pulmonary arteriovenous fistula reviewed by Weiss and Gasul,[6] approximately one half of the patients gave a history of telangiectases in other members of the family, over one half had telangiectases of the skin or mucous membranes, and over one third had more than one such lesion within the lungs.

In the reported cases in which the pathologic findings have been described in any detail, interest has been focused on the large saccular fistulae, although many writers mention the presence of other smaller lesions in the same lung. It is remarkable that only 7 cases have been reported in which the classical triad of cyanosis, digital clubbing, and polycythemia has resulted from the presence of multiple small pulmonary lesions in the absence of any large lesion of the saccular type.[5-11] The apparent rarity of this form of the disease may reflect the difficulty of clinical diagnosis when the lesions are too small to be demonstrated by chest films or angiograms. It may also be due to the difficulty in recognizing and demonstrating the lesions anatomically by the usual gross dissection and microscopic examination.

This report concerns 2 cases of multiple small pulmonary arteriovenous fistulae studied post mortem by the injection-corrosion cast technique. In neither case was the correct diagnosis established definitely during the patient's life. In the first, the clinical diagnosis was congenital heart disease. In the second, the correct diagnosis was made and adhered to by several clinicians even though it could not be confirmed by angiograms, exploratory thoracotomy, or lung biopsy. Besides illustrating that the typical clinical picture of pul-

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monary arteriovenous fistula can be produced by multiple tiny lesions as well as one or several large ones, the bronchovascular casts demonstrate the varying structure of these small pulmonary lesions—from complex tiny vascular plexuses characteristic of the telangiectases elsewhere to miniature replicas of the saccular arteriovenous fistulae commonly described in the lungs.

Case 1

The patient, a white boy, 17 years old, was admitted to the Los Angeles County Hospital for the only time 5 hours before his death. A diagnosis of purulent meningitis had been established at another hospital. By the mother's report, the patient had been quite well until 8 years of age, when he began to have frequent nosebleeds. During the following year he had a posterior pharyngeal hemorrhage and a severe episode of rectal bleeding. Cyanosis and exertional dyspnea became manifest during the tenth year of life and persisted thereafter. The digits became clubbed and episodes of fainting occurred. A diagnosis of congenital heart disease was made by several physicians although cardiac fluoroscopy was the only special diagnostic procedure employed. The mother had never noted any lesions of the patient's skin or lips which might be interpreted as telangiectases. No other members of the family had been known to have such lesions, or to have had epistaxis, hemoptysis, or melena. The parents had been divorced for several years so knowledge of the health of paternal relatives was limited.

Physical examination revealed the typical signs of acute meningitis. Petechiae were described on the chest, face, and lips. The lungs were clear to percussion and auscultation. The heart was believed to be enlarged and a loud systolic murmur was heard over the entire precordium. The nailbeds were cyanotic and the digits were markedly clubbed. Hemoglobin was 13.5 gm. per cent. Gram-negative diplococci were seen in the purulent cerebrospinal fluid, but cultures were sterile. Death occurred approximately 5 hours after admission.

Gross Post-mortem Examination. Necropsy, performed 13 hours after death by Dr. Bruce Wallace, confirmed the diagnosis of purulent meningitis. A fading petechial rash was present over the face, chest, and extremities, but no definite cutaneous telangiectases could be found. The heart weighed 375 gm., and all chambers appeared slightly dilated and hypertrophied. The major pulmonary arteries were believed to be slightly dilated also. No congenital abnormalities were found in the heart or great vessels. The surfaces of the lungs showed numerous tiny, round, red subpleural lesions resembling petechial hemorrhages (Fig. 1). Close examination, however, revealed that these were dense plexuses of tiny vessels. The lesions ranged from 2 to 5 mm. in diameter and usually were associated with focal accumulations of anthracotic pigment. Partial dissection of the pulmonary vessels failed to disclose any large arteriovenous fistulae, so the lungs were inflated with 5 per cent formalin and saved for injection-corrosion studies. There were no other significant visceral lesions. The gastro-

intestinal tract was searched carefully for mucosal telangiectases, but none was found.

Bronchovascular Corrosion Cast. The method of preparing the bronchovascular corrosion cast was essentially that described by Liebow *et al.*[12] Previous partial dissection of the lungs necessitated separate cannulation of many small vessels and bronchi. The plastic, a 12.5 per cent acetone solution of vinylite and diatomaceous earth, was injected by syringe after first clearing the vessels with air and acetone. Although the vinylite solutions were known to be too viscid to penetrate capillaries, there was a free flow of the plastic from pulmonary arteries to veins and vice versa, confirming the existence of sizable arteriovenous anastomoses. Bronchi also were injected, and after the plastic had solidified the tissue was digested in concentrated acid. The resultant cast was washed in running water and then defatted in petroleum ether.

The cast of each lung was studded by several hundred discrete plexuses of tiny vessels connecting dilated peripheral pulmonary arteries and veins (Fig. 2). The plexuses ranged from 1 to 10 mm. in diameter. They were largest and most numerous at the pleural surface, but were present also deep within the lung. Distribution was quite uniform in the different lobes and segments. The larger lesions were roughly spherical, supplied by several arteries at one hemisphere, and drained by several veins from the opposite hemisphere (Fig. 3). Large peripheral lesions often were flattened on the pleural surface (Fig. 4). These tended to have a stellate appearance, with channels radiating toward a centrally situated vein. This central vein was never the sole means of venous drainage of the plexus. In the plexuses, the vessels usually were quite uniform in diameter, but occasionally one of the vascular limbs was appreciably larger than the rest (Fig. 4). Vessels were so numerous and so closely approximated in the lesions that only those located peripherally could be traced throughout their tortuous course from artery to vein. Smaller plexuses of 1 to 3 mm. in diameter had the same general structure but the communicating vessels were fewer and often less tortuous (Fig. 5). Some consisted of no more than two or three vessels easily traced under the dissecting microscope. In the left posterior basal segment there was a direct aneurysmal communication between a single 2 mm. artery and vein (Fig. 6). Throughout the cast, lobar and segmental arteries and veins appeared only slightly dilated. However, in smaller vessels near the plexuses, dilatation was conspicuous

and was generally more severe in arteries than in veins. Vessels frequently had bulbous expansions just before origin of the smaller channels supplying or draining the plexuses. No attempt was made to identify and inject bronchial vessels; they were not injected retrogradely from either pulmonary arteries or veins.

Histologic Observations. Sections of lung were stained with hematoxylin and eosin and elastica-van Gieson's stains. The ten sections examined from each lung included a total of eighteen plexuses. Larger lesions resembled cavernous hemangiomas (Fig. 9); numerous small vessels, most in the range of 75 to 125 μ, lay in immediate apposition, separated only by their thin walls and small amounts of connective tissue. In smaller lesions, many vessels were separated by well expanded, normal appearing alveoli (Fig. 11). Walls of the small vessels of the plexuses were composed of endothelium resting on a layer of hyaline connective tissue of variable thickness. The latter occasionally contained a few short elastic fibers, but there was no evidence of smooth muscle. Larger arteries and veins were remarkably dilated, particularly those near the vascular lesions. Variation in the thickness of the walls of these vessels was a consistent feature (Fig. 10). Elastic stains indicated that the focal thickening was due to intimal fibrosis; the undue thinning, to loss of muscle and elastic tissue in the media. A few lymphocytes and carbon-filled macrophages often were present in and around the lesions, and rarely an adjacent alveolus contained a few phagocytes with brown pigment. No old or recent thrombi were found. Because of their similar size, the angiomatoid lesions strongly resembled focal alveolar hemorrhages on casual examination. In histologic sections of many of the other viscera there was rather marked dilation of small arteries and veins, but only in the random section of parathyroid gland was a discrete angiomatous lesion found. This was a 2 mm. collection of tiny, thin-walled vessels, similar to the pulmonary lesions except for the generally smaller size of the component vessels.

Case 2

The patient, a white woman, 30 years old, had not been well since the age of 18 years, when she had an illness characterized by recurrent jaundice and followed by anemia. At the age of 23 years, she was found to have an enlarged spleen, which was subsequently removed. Several months later there occurred the first of many episodes of coma, each lasting for several days. She was first seen at the Los Angeles County Hospital at the age of 27 years during her last episode of coma. As had been the case in other hospitals, cerebral angiograms and ventriculograms were negative. A previous diagnosis of hypothyroidism was confirmed. Endometrial biopsies indicated very low estrogen effect, and 17-ketosteroid excretion was subnormal. Tests of liver function were all abnormal. Apparently for the first time,

the patient was found to have clubbed digits and to be cyanotic and polycythemic. During the subsequent 3 years the mechanism of the cyanosis was investigated extensively at the Los Angeles County Hospital and the Hospital of the Good Samaritan. Cardiac catheterization revealed normal pulmonary arterial pressure with no evidence of an intracardiac shunt. Studies of pulmonary function were normal except for evidence of arteriovenous shunting; arterial oxygen saturations were in the range of 70 to 80 per cent. Angiograms on three separate occasions failed to reveal pulmonary arteriovenous fistulae, although there was a faint haziness at the left base on the routine chest film and two examiners heard a questionable bruit there. Eventually left thoracotomy was performed, but no arteriovenous fistulae could be identified. Random biopsies of the lingula and lower lobe at that time were characterized by focal interstitial pneumonitis and a moderate degree of arteriosclerosis of the small arteries; there were no lesions suggestive of arteriovenous fistulae in the histologic sections. During the subsequent 10 months until the patient's death, liver function continued to deteriorate, and a slight macrocytic anemia replaced the polycythemia. She expired suddenly at home during an episode of acute respiratory distress.

During the last 3 years of the patient's life, spider angiomas were noted frequently on the skin. No cutaneous lesions of the Rendu-Osler-Weber type were seen, but one examiner described a discrete "hemangioma" on the lower lip. Epistaxis occurred with several upper respiratory infections, and slight diffuse telangiectasia of the nasopharyngeal mucous membrane was described. There had never been any sign of gastro-intestinal or pulmonary hemorrhage. No family history suggestive of telangiectasia was obtained.

Gross Pathologic Examination. Necropsy was performed approximately 18 hours after death. Telangiectases could not be identified on the skin, lips, or oral mucous membranes. The digits were severely clubbed. The chambers of the heart, which weighed 300 gm., were dilated, particularly the right ventricle and right atrium. The pulmonary conus was prominent and the major pulmonary arteries were dilated. There were no congenital abnormalities of the heart or great vessels. Surfaces of the lungs were partially obscured by thickened adherent parietal pleura; arteriovenous fistulae could not be seen. In the cirrhotic liver, large regenerated nodules alternated with wide bands of atrophy and fibrosis. The thyroid gland weighed only 10 gm. and appeared entirely fibrous; other endocrine glands were not remarkable grossly. Ventriculography wounds were present in each occipital cortex, and tiny foci of encephalomalacia and old hemorrhage were found in locations compatible with needle tracts.

Bronchovascular Cast. While the lungs were inflated with positive intratracheal air pressure, the main pulmonary artery was injected with a 12 per cent acetone solution of vinylite and diatomaceous earth. A free flow of plastic from the pulmonary veins confirmed the existence of gross arteriovenous anastomoses. The left atrium and pulmonary veins were then injected with a similar mass of different color. When the plastic had solidified in the vessels, the trachea and bronchial tree were injected with an uncolored mass. After digesting

the tissue in acid, the cast was washed in running water and defatted in petroleum ether.

Five discrete vascular lesions were visible in the cast. The largest of these was a roughly spherical plexus beneath the costal pleura of the left posterior basal segment (Fig. 7). It measured 12 mm. in diameter and consisted of tiny tortuous vessels measuring 0.5 to 1.0 mm. in diameter. It was supplied by three, 1 to 2 mm., dilated arterial branches cupped over one hemisphere and drained by two smaller tributaries of a 2.5 mm. bulbous pulmonary vein. Normally arborizing branches arose from the larger vessels supplying and draining the plexus. A 7 mm. plexus of smaller vessels was situated only a few centimeters away. On the diaphragmatic pleural surface of the right anterior basal segment there were three loosely grouped, tortuous vessels of 0.2 to 1.0 mm. diameter connecting branches of a dilated artery and vein. An even more simple plexus of slightly larger vessels was present on the medial aspect of the left anterior basal segment.

On the diaphragmatic aspect of the right anterior basal segment there was an arteriovenous fistula of rather different structure from those just described (Fig. 8). It consisted of a 2 mm. vessel communicating after five close U-shaped bends with a single artery and vein. Surrounding this extremely simple and direct arteriovenous anastomosis were several small, tortuous vessels establishing similar but more complex arteriovenous fistulae. The general structure here suggested a smaller spherical plexus in which one limb had undergone extreme dilatation.

Although the five lesions just described were the only ones in which actual arteriovenous anastomoses could be identified, it is probable that many smaller lesions were not injected due to an unexpectedly high viscosity of the injection mass. Contributing to this impression were remarkably dilated distal arteries and veins. These were most striking at the costal-diaphragmatic margin of the right lower lobe where several tortuous pulmonary arteries actually increased in diameter as they approached the pleural surface; their branches ramified beneath the pleura and communicated with other pulmonary arterial branches. No bronchial vessels were injected retrogradely from either pulmonary arteries or veins.

Histologic Observations. The sixteen random sections of lung included two angiomatous lesions. They were similar to those described in the previous case except that the vessels of the plexuses were larger and their walls contained somewhat more elastic tissue. Between the vessels there was an infiltrate of lymphocytes, plasma cells,

and large mononuclear cells. Pulmonary arteries and veins were conspicuously dilated and in their walls focal intimal thickening alternated with marked thinning of the media. As in the previous case, these changes were more striking in the arteries than in the veins. Focal interstitial fibrosis and round cell infiltration were noted in many sections, but arteriosclerosis was much less prominent than in the biopsy specimens. The pleural adhesions were extremely vascular. The sections of ovary included three discrete, 4 to 5 mm., angiomatous plexuses of thin-walled vessels ranging from capillary size to 1 mm. in diameter. Primordial and developing follicles were extremely rare in all ovarian sections. No angiomatous lesions were found in sections of other viscera. Histologic findings in the liver were compatible with the gross impression of post-necrotic cirrhosis. The thyroid gland was almost entirely replaced by dense fibrous connective tissue. Cerebral lesions definitely attributable to acute or chronic anoxemia were not found.

Discussion

Since atavism may mask the hereditary nature of the disease,[2,13] there is justification for classifying these cases as variants of hereditary hemorrhagic telangiectasia even in the absence of a positive family history. Each patient had proved lesions in at least one site other than the lungs, and the first patient undoubtedly had undiscovered lesions of the mucous membranes which were responsible for epistaxis and intestinal bleeding. The clinical history of this boy is typical of many reported cases of pulmonary arteriovenous fistulae complicating hereditary hemorrhagic telangiectasia; epistaxis and intestinal bleeding began in childhood and were followed by the development of cyanosis and clubbing of the digits. Even the incorrect diagnosis of congenital heart disease might be considered typical. The history of the second patient is somewhat bizarre because of hepatic and endocrine disorders which preceded by several years the typical clinical manifestations of the pulmonary vascular lesions.

Anatomically, the lesions of these two cases conform to Bean's[14] description of the telangiectases of Osler's disease as "... tortuous coiled masses of tiny aneurysmal vessels including capillaries and small veins and arteries." They also conform to the description of arteriovenous fistulae of the lung by Moyer and Ackerman.[15] These authors, in summarizing the cases reported prior to 1948, stated that "between the arterial and venous systems there is either a direct communication through one or several large vascular trunks, or a tangle of more or less distended vessels instead of capillaries."

Part of the interest in the two cases described here lies in the presence of lesions intermediate between the tiny spherical plexuses characteristic of hereditary hemorrhagic telangiectasia and the multiloculated saccular fistulae commonly described in the lungs. They indicate that the large saccular lesions may arise by progressive dilation of one or several "favored" limbs of a complex spherical plexus. The lesion in Figure 4 illustrates the first stage of such a transformation; one of its limbs is appreciably larger than the rest. The lesion in Figure 8 is a further step in the process; a large multicoiled vessel is almost all that remains of the original plexus; most of the other limbs have disappeared, conceivably due to diversion of blood into the larger channels of lesser resistance. Dilatation to this degree incorporates the proximal artery and distal vein into the fistula itself, and normally arborizing vessels may thus appear to arise from the fistula; this was noted in several of the larger lesions here and was described also in saccular aneurysms by Lindskog, Liebow, Kausel, and Janzen.[16] Further dilatation without elongation of the vessel in Figure 8 could be expected to bring its separate loops or coils into close apposition, with the formation of a multiloculated sac. The casts prepared by Lindskog and his co-workers demonstrate that the multiloculated appearance of the large saccular aneurysms results from dilatation and confluence of separate coils and loops. Separate vascular limbs of the plexus may conceivably rupture into each other and contribute further to the final multiloculated, saccular appearance, but no gross or histologic evidence of such rupture could be found in the lesions described here. Simple vascular dilatation alone seems adequate to explain the transformation from complex tiny plexuses to large saccular fistulae. That such transformation in hereditary hemorrhagic telangiectasia occurs only in the lung may be due to the fact that only in the lung is tissue resistance sufficiently small to allow such massive vascular dilatation.

Although a strong genetic component in hereditary hemorrhagic telangiectasia is well established, the fundamental pathogenesis remains obscure. The two cases described here contribute little in this regard except to support the concept that the primary vascular dilatation involves vessels with the anatomical structure and location of capillaries. On the basis of promising results using rutin in the treatment of hereditary hemorrhagic telangiectasia, Kushlan[17] has suggested that the vascular dilatation may be a manifestation of a generalized weakening of ground substance, which is due in turn to a defect in the normal hyaluronidase-inhibiting mechanism. That there

may also be an endocrine component in the development of the lesions is indicated by the report of Koch, Escher, and Lewis.[18] These workers noted that in women with hereditary hemorrhagic telangiectasia, epistaxis appeared to be related to periods of low estrogen secretion and high urinary gonadotropin excretion; and furthermore, the administration of estrogens resulted in marked diminution in the frequency and severity of the hemorrhages, and in actual reduction in the size and number of the lesions of the skin and mucous membranes. In this regard it is worthy of note that the second case described here had evidence of subnormal estrogen secretion. The inhibitory effect of estrogens on the lesions of hereditary hemorrhagic telangiectasia indicates another major difference between these lesions and the angiomas of cirrhosis and pregnancy, for the latter are closely related to increased rather than decreased estrogen levels. Bean[14] has pointed out significant anatomical differences between these two lesions, mainly that spider angiomas are composed of dilated arteries draining into normal capillaries rather than dilated vessels forming true anteriovenous anastomoses.

The two cases described here present several significant features in regard to the clinical and pathologic diagnosis of small pulmonary arteriovenous fistulae. In spite of the availability of a typical clinical history, the correct diagnosis was not made in the first case, presumably because it was never considered. However, in the second case, with an atypical history, the correct diagnosis was made after the essential cardiac and pulmonary studies had been performed and the possibility of other right-to-left shunts had been excluded. The consistently negative angiograms of this patient indicate the limitations of this procedure in visualizing the small lesions. Only in the case reported by Sacrez and co-workers[11] have multiple tiny arteriovenous fistulae been demonstrated by this method. Thoracotomy and pulmonary biopsy established the correct diagnosis in the case reported by Cooley and McNamara,[10] and might have been equally successful in the first case reported here. However, when the plexuses are relatively few and are not visible on the pleural surface, as in the second case, random biopsy procedures would seem to have little chance of success. The presence of moderately severe arteriosclerosis of small pulmonary arteries, as was noted at biopsy in this patient, does not contraindicate the possibility of arteriovenous fistulae in the same lung. Ingleby described similar arteriosclerotic lesions in Behrend and Baer's[7] case, and attributed them to disuse.

The value of the injection corrosion technique for the post-mortem

or postoperative demonstration of vascular shunts is exemplified particularly in the second case. It is doubtful if the correct clinical diagnosis could have been substantiated in any other way.

Summary

Two cases of multiple small pulmonary arteriovenous fistulae are reported. Both patients had lesions in at least one other organ, and although there is no family history of the disease, both are considered to be examples of hereditary hemorrhagic telangiectasia or Rendu-Osler-Weber disease. In vinylite injection-corrosion casts of the lungs, the lesions consisted of dense plexuses of tiny vessels connecting dilated pulmonary arteries and veins. Lesions intermediate in type between plexuses and saccular aneurysms indicate that the latter may arise by progressive dilatation of one or several limbs of a small plexus. Some of the etiologic and diagnostic considerations in the disease are discussed briefly.

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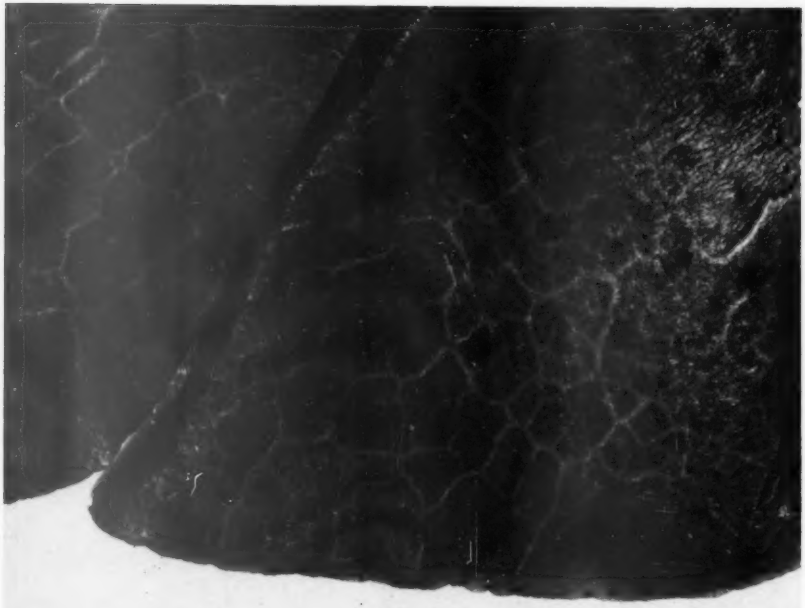
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 1. Pleural surface of the left lower lobe and lingula. The blood has been washed out of most of the plexuses, but several are still visible as round foci where dark pigment has been concentrated. × 1.25.

FIG. 2. Case 1. The pleural surface of the bronchovascular corrosion cast is studded with numerous plexuses of tiny vessels communicating between dilated peripheral arteries and veins. The arrows point to three of the larger lesions in this small field. × 3.



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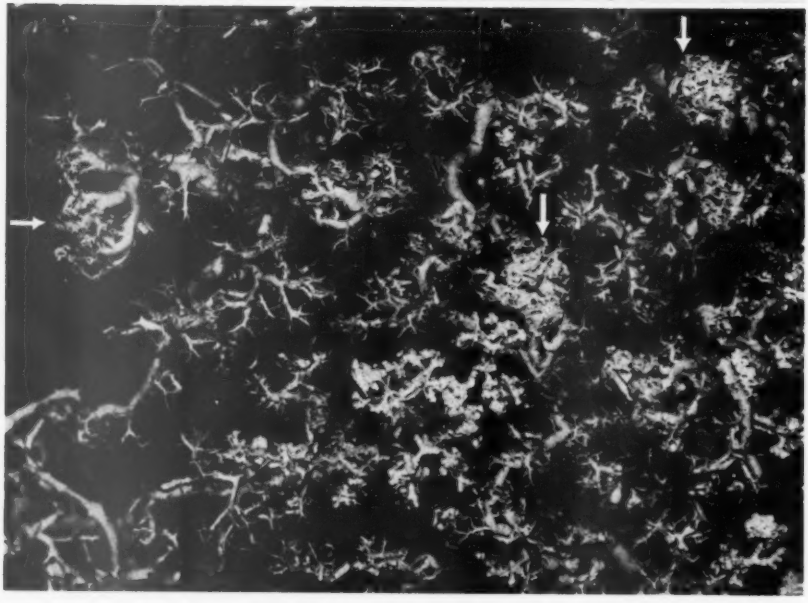

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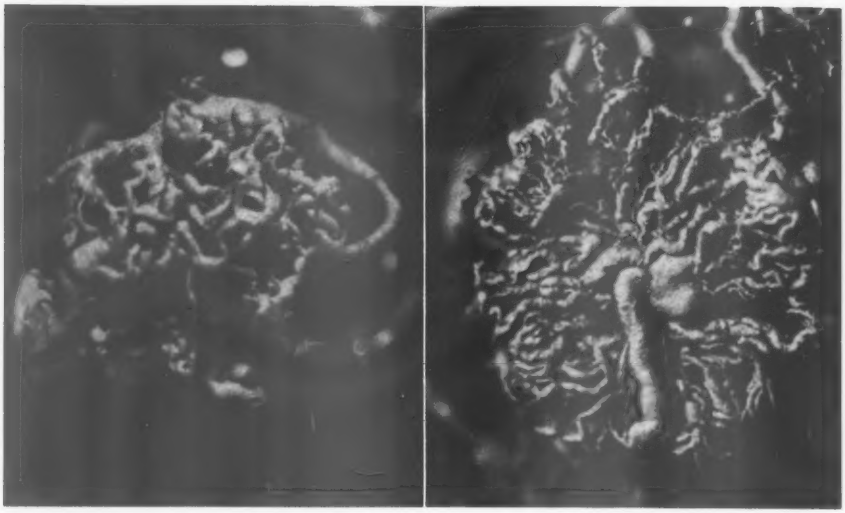

3 4

FIG. 3. Case 1. Several branches of a dilated artery supply this spherical plexus from the upper right. Several branches of a dilated vein drain it from the lower left. × 25.

FIG. 4. Case 1. A peripheral plexus is flattened on its pleural surface. It has a stellate appearance, with vessels converging toward a central vein. Several separate arteries supply the plexus from its periphery and, although not apparent here, several separate veins drain it from the opposite side. One limb of the plexus is remarkably enlarged as compared to the rest. × 14.

FIG. 5. Case 1. One large and two small plexuses viewed from their venous side. Arteries are on the other side and are out of focus. × 20.

FIG. 6. Case 1. A direct arteriovenous fistula has a bulbous expansion at the actual site of arteriovenous anastomosis. The artery is below, the vein above. × 4.

FIG. 7. Case 2. This spherical fistulous complex was located near the pleura on the posterior surface of the left lower lobe. Three dilated arteries are cupped over a tortuous rete of smaller vessels. On the left, one of the venous tributaries drains into an abruptly dilated pulmonary vein. × 5.7.

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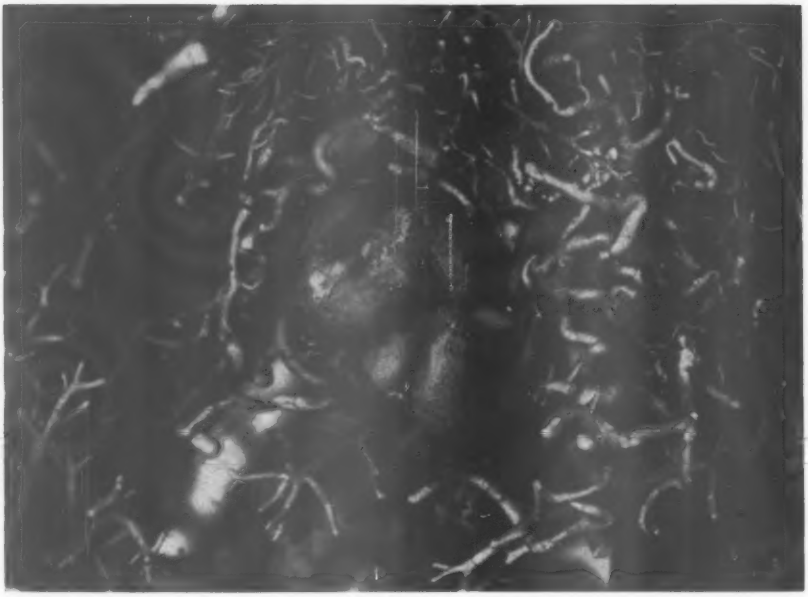

FIG. 8. Case 2. Three of five sharp, U-shaped bends are visible here in this very simple and direct arteriovenous fistula. Surrounding this vessel are several small, tortuous, arteriovenous anastomoses, suggesting that the entire lesion was originally a spherical plexus in which one limb has become greatly dilated. Further dilatation without further elongation could conceivably convert the large vessel into a typical multiloculated saccular aneurysm. × 4.5.

FIG. 9. Case 1. A large plexus has the histologic structure of a cavernous hemangioma. Most of its vessels are approximately the size of alveoli, and under low magnification such a lesion may easily be mistaken for a group of alveoli containing extravasated blood. × 16.5.

FIG. 10. Case 1. There is great variation in the thickness of the walls of the dilated pulmonary blood vessels. Little or no muscle or elastic tissue persists in the media. In focal zones the intima is greatly thickened by fibrosis. Such changes in the vessel walls often make histologic distinction between arteries and veins impossible. × 80.

FIG. 11. Case 1. Diatomaceous earth from the injection mass distends the vessels of a small plexus. Expanded alveoli separate many of the vascular limbs. A pulmonary artery is situated below the empty bronchus. A vein draining the plexus is partially included at the right margin. × 50.

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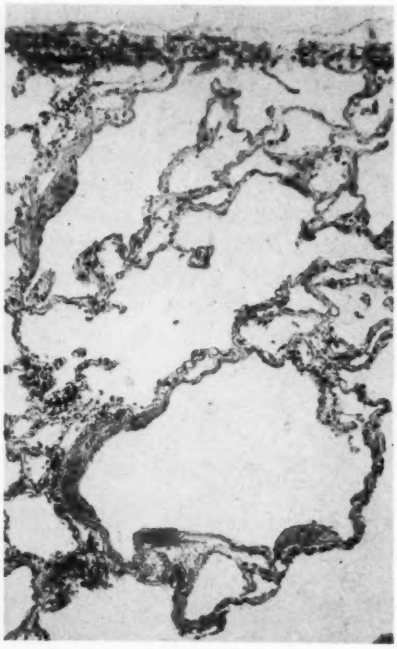

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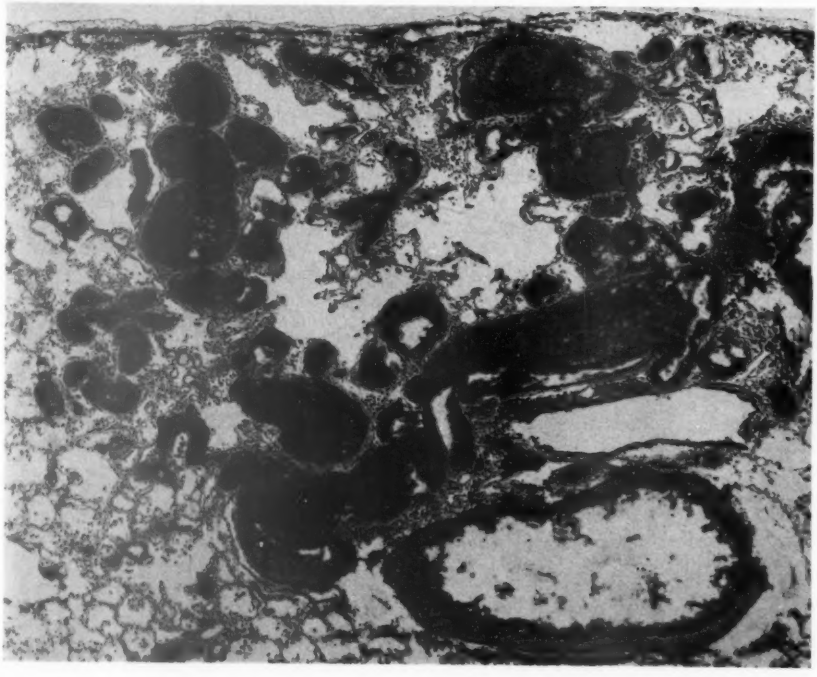

11

PERIODIC ACID-SCHIFF-POSITIVE RETICULO-ENDOTHELIAL CELLS PRODUCING GLYCOPROTEIN

Functional Significance During Formation of Amyloid*

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The present study deals with investigations on functional stages of reticulo-endothelial cells characterized cytochemically by the presence in their cytoplasm of a polysaccharide-containing substance which is colored by the periodic acid-Schiff (PAS) technique.

In response to various noxious stimuli, a proportion of proliferating reticulo-endothelial cells (including plasma cells, reticulum cells, the Kupffer cells of the hepatic sinusoids, the endothelial cells of the renal glomerular tufts, endothelial and adventitial cells of vessels) were found to contain granular or globular PAS-positive material in the cytoplasm (PAS cells).

PAS cells, pyroninophilic cells, and intermediate cell types containing PAS-positive as well as pyroninophilic material in the cytoplasm are considered characteristic functional stages of reticulo-endothelial cells involved in the morphogenesis of a variety of morphologic lesions of mesenchymal tissue.

Evidence is presented that PAS cells are linked directly with the synthesis of amyloid and related substances. Apart from accounting for the local cellular secretion of polysaccharide-containing globulins during the formation of amyloid, which is known to be a glycoprotein, the findings may also provide a reasonable explanation for the associated abnormalities in the serum protein-bound polysaccharides.

ETIOLOGIC THEORIES ON THE CAUSATION OF AMYLOIDOSIS

Amyloidosis and paramyloidosis are characterized morphologically by deposition of a homogeneous, non-fibrillar, hyaline-like substance for which certain tinctorial methods are specific. Hass and his coworkers[1] found that amyloid is formed by two slightly different protein fractions and a sulfate-bearing polysaccharide in secondary amyloidosis, and showed that from 1 to 2 per cent of the amyloid molecule is of carbohydrate nature. It was suggested that the physicochemical properties of amyloid are not contributed by the

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protein alone, and that components of the circulating fluid may be bound to this matrix. Amyloid of different species and from different sources may show variation in composition and staining properties. The basic etiologic theories have been reviewed recently.[2-4] Since amyloid is related chemically to the interfibrillary ground substance of connective tissue, it has been suggested that the excessive breakdown of connective tissue might release into the blood stream components of ground substance in soluble form, capable of precipitation elsewhere as amyloid. Several authors have considered amyloidosis an infiltration rather than a degeneration. Pirani[5] postulated that in conditions in which depolymerization of the ground substance occurs and in which raised glycoprotein levels are maintained for a sufficiently long period, glycoproteins will precipitate at the level of the reticulo-endothelial system. Faber,[6] who observed an increase in the serum glucosamine associated with suppurative infectious disease, suggested that the serum mucoid was deposited in the tissue as amyloid. It has been pointed out[7] that, like mucin, amyloid is a glycoprotein, and a relationship to the mucolysis that occurs in the joints in rheumatoid arthritis has been suggested.

An alternative hypothesis, put forward by me,[2] accounts for the association of amyloidosis, not only with rheumatoid arthritis, but with the other conditions in which it is frequently found. This theory is founded upon histologic and experimental studies of reaction of mesenchymal and reticulo-endothelial cells in various conditions, and the control of the protein-synthesizing function of the reticulo-endothelial system and its pyroninophilic derivatives, the plasma cells and their immediate precursors, by the actions of adrenal corticoids and ascorbic acid. The breakdown of this control in persistent antigenic stimulation or other form of stress results in the local formation of amyloid.

The studies on experimental amyloidosis showed evidence of inhibition of cellular proliferation in the reticulo-endothelial apparatus, probably associated with a disturbance of some enzyme system to account for amyloid formation. Injections of cortisone or corticotrophin induced or promoted amyloid deposition in mice treated with injections of sodium caseinate,[2] and under the same experimental conditions three injections of nitrogen mustard (NH_2)—each equivalent to from 2.5 to 5 mg. per kg. of body weight—induced an almost diffuse amyloid deposition in the spleen.[8] On the other hand, ascorbic acid maintained the pyroninophilic cellular reaction in the spleen of hyperimmunized rabbits.

The experimental results with cortisone and ACTH were confirmed by Latvalahti[8] (1953), and clinical observations of amyloidosis following treatment with cortisone (West and Newns,[9] 1952) or ACTH (Frenkel and Groen,[10] 1954) have been reported.

The active stage of mesenchymal diseases[11] and experimental conditions produced by repeated stimulation of immune mechanism[12] often were associated with a proliferation of plasma cells and pyroninophilia of the cytoplasm of proliferating reticulo-endothelial cells in the spleen, liver, renal glomeruli, adventitia of vessels, and capillary endothelium. Moreover, this active phase was characterized by elevated levels of serum γ-globulin. Treatment with cortisone exerted a marked depression of pyroninophilia and plasma cells and, under certain conditions, caused a local precipitation of a pale, homogeneous substance of a type conforming to some or all of the morphologic criteria of amyloid. Amyloid formation is associated with changes in the electrophoretic pattern of the serum proteins. This was studied in mice by Letterer[13] (1949) and by Bohle and co-workers[14] (1950); following from 12 to 20 injections of nucleic acid, they found unquestionably elevated levels of γ-globulin, followed by a fall in γ-globulin and a rise in the levels of α- and β-globulin. The animals which developed amyloidosis after 30 injections had lower levels of γ-globulin than those which remained unaffected.

Whereas plasma cells are concerned in the formation of antibodies in response to antigenic stimuli, the predominant rôle of plasma cells and other pyroninophilic mesenchymal cells in the acute stage of ascorbic acid deficiency[15] and in other conditions in which evidence of persistent antigenic stimulus is lacking is remarkable. Pirani and co-workers[16] observed marked amyloid deposition in guinea-pigs fed on a scorbutogenic diet for 8 weeks or longer. In post-mortem examinations comprising 28 cases of rheumatoid arthritis, Lindahl and I[17] found an exceedingly high incidence of amyloidosis *histologically* (17 of 28 cases); we considered amyloid deposition in the spleen, kidney, and other organs to be a characteristic histologic lesion in long-standing rheumatoid arthritis and to be much more frequent than suggested by approximately 100 cases (cf. Reece and Reynolds[18]) reported in the literature.

The association between rheumatoid arthritis and amyloidosis is of the greatest interest for the light it might throw on the pathogenesis of these two disorders. Altogether, the common essential factors involved in the pathogenesis of rheumatoid arthritis, amyloidosis, and general stress phenomena may account for the exceedingly high inci-

dence of amyloid lesions in rheumatic disease. Actually, amyloidosis and the preceding cellular reaction represent a characteristic phase in reticulo-endothelial cellular function following stress.[15,17] In this connection, attention may be drawn also to the observations of plasmacytosis in such conditions as atomic energy casualties (Liebow and co-workers,[19] 1949), chronic radium poisoning in rats (Thomas and Bruner,[20] 1933), and x-ray irradiation in dogs (Wohlwill and Jetter,[21] 1953). The studies of Lundin *et al.*[22] (1954) indicate that plasma cell proliferation is a general reaction promoted by some pituitary factor which is secreted in increased quantities during adaptation; subsequent studies from the same laboratory (Schelin *et al.*[23]) showed this to be the somatotrophic hormone.

PREVIOUS OBSERVATIONS OF PAS-POSITIVE SUBSTANCE IN RETICULO-ENDOTHELIAL CELLS

Among the oxidation methods, the periodic acid-Schiff technique (McManus,[24] 1946; Lillie,[25] 1947; and Hotchkiss,[26] 1948) has become extensively used in histochemical procedures. There seems to be fairly complete agreement that 1,2-glycols are demonstrated in tissue sections by the PAS technique, and that these are most numerous in material consisting of carbohydrate or containing a carbohydrate moiety. These methods have been used especially in various researches on intercellular substances in mesenchyme; but, apart from histochemical studies by Pearse[27] (1949), White[28] (1954), and Grundner-Culemann and Diezel[29] (1955) on the nature of Russell bodies and their relation to plasma cells, only slight attention has been paid to the significance of mesenchymally derived PAS-cells and their possible rôle in a variety of lesions examined in the present study.

Pearse[27] (1949) used the periodic acid-Schiff method of McManus and Hotchkiss, together with other techniques, to investigate the cytochemistry of Russell bodies in human plasma cells and of Kurloff bodies in lymphocytes of guinea-pigs, and presented evidence that both consist of mucoprotein. The substance of which the bodies are composed was found uninfluenced by ribonuclease, diastase, or hyaluronidase; it failed to bind methylene blue at pH 6, and was devoid of metachromatic properties. It also was demonstrated that the cytoplasm of some normal plasma cells contains mucoprotein, suggesting that these are polysaccharide-containing globulins (glycoglobulins, mucoglobulins). Pearse examined 6 cases of plasmacytoma, and found a PAS-positive reaction in a small proportion of plasma cells in 4, while in 2 cases a large proportion of the plasma cells and reticulum

cells showed a positive reaction. In tissue in which plasma cells containing Russell bodies were present in addition to the ordinary types, Pearse observed intermediate stages between the plasma cells with faintly PAS-positive cytoplasm and those containing the fully developed Russell bodies. Pearse considered the question whether the mucoprotein is secreted or absorbed by plasma cells, and adduced evidence in favor of secretion; this is in accord with my assumption[11] (1948) that plasma cells and other pyroninophilic reticulo-endothelial cells may produce amyloid and hyalin.

Cavallero[30] (1953), who made a morphologic study of the effect of somatotrophic hormone on the plasma cells, found that treatment of intact rats with this hormone resulted in an increasing number of plasma cells in the spleen, lymph nodes, and thymus. By the periodic acid-Schiff method abundant granular PAS-positive material was demonstrated in the immature plasma cells and in undifferentiated reticulum cells as well as in the interstitial spaces. Russell bodies frequently were seen in the lymph nodes.

Present Study

The periodic acid-leukofuchsin procedure of McManus and Hotchkiss was used to examine the cytologic changes in reticulo-endothelial and other cells derived from mesenchyme, which were previously found to be linked with the formation of amyloid and related substances.

MATERIAL AND METHODS

Five different groups, with a total of 80 female mice of C_3H strain, weighing from 15 to 25 gm., were fed ad libitum on an oatmeal diet during the experiment. The animals were given daily subcutaneous injections of 0.5 ml. of a 5 per cent casein solution in 0.25 per cent NaOH for periods varying from 28 to 36 days. In 25 animals the casein injections were followed by four daily subcutaneous injections of 0.3 mg. of cortisone, whereas 10 animals were treated with 0.5 mg. of ACTH in two daily doses. Another 16 mice received three injections of nitrogen mustard (NH_2), each equivalent to from 2.5 to 5 mg. per kg. of body weight.

A group of 48 rabbits were hyperimmunized with a formaldehyde-killed Pfeiffer bacillus culture, administered in intravenous injections three times a week for from 6 to 16 months (cf. the material previously used for studies on the effect of cortisone on plasma cell response[31] and in studies on the effect of cortisone on experimental glomerulonephritis.[12])

A group of 5 guinea-pigs were treated with daily subcutaneous injections of 2 ml. of a 5 per cent casein solution in 0.25 per cent NaOH for 5 weeks.

Specimens of various organs were fixed in 4 per cent formalin and examined histologically after staining with periodic and leukofuchsin —McManus (PAS) and Hotchkiss (PARS)—the Unna-Pappenheim methods, and with methyl violet and Congo red stain for amyloid.

DESCRIPTION OF FINDINGS

Experimental Amyloidosis in Mice

The earliest and most pronounced changes were observed in the *spleen*. Histologic examination showed increasing accumulations of plasma cells and highly pyroninophilic reticulum cells, especially in the red pulp; this also contained numerous giant cells with three or more nuclei in irregular arrangement.

Sections stained with the PAS technique showed, during treatment, an increasing number of proliferating reticulo-endothelial cells showing PAS-positive, often finely granulated material in the cytoplasm (PAS cells); also, after administration of casein for some weeks, the cytoplasm of giant cells showed globular PAS-positive inclusions. Comparison with sections stained by the Unna-Pappenheim method showed intermediate cell types containing a pyronine-positive zone of cytoplasm surrounding inclusions of non-pyroninophilic, PAS-positive substance. Such intermediate cell types were visible especially among the giant cells, presenting a decreasing amount of pyroninophilic material in the cytoplasm as the amount of PAS-positive substance increased. The pyroninophilia was removable by incubation with ribonuclease, whereas the PAS-positive substance remained uninfluenced by ribonuclease, diastase, or hyaluronidase, failed to bind methylene blue at pH 6, and was devoid of metachromatic properties.

Similar cytochemical changes were observed in relation to the cells of the splenic reticulum in the perifollicular zone and in endothelial and adventitial cells of vessels showing PAS-positive material.

In all cases it was evident that the PAS-positive reticulo-endothelial cells were directly linked with amyloid formation and deposition locally. This was especially pronounced in cases in which amyloid production was accelerated for a short period by additional administration of ACTH, cortisone, or nitrogen mustard. In this stage of intensive amyloid formation accompanied by gradual suppression of the cellular elements of the spleen, accumulations of large reticulum cells, showing bright red cytoplasm when stained with the PAS tech-

nique, in direct continuity with the amyloid precipitates were distinctive findings (Fig. 1). The observation in the border zone of large, hypertrophic PAS cells showing signs of secretion of a homogeneous material confluent with the amyloid precipitates (Fig. 2) showed strikingly the functional significance of reticulum cells in the secretion of glycoprotein during the local precipitation of amyloid. More scattered giant cells with PAS-positive cytoplasm also showed signs of a direct transformation into small isolated deposits of amyloid. Analogous changes in the splenic reticulum and vascular endothelium accounted for the amyloid deposition in relation to these structures. When the amyloid deposition was almost complete, the pyroninophilic cellular reaction was found to be suppressed, but a few reticulum cells with a strongly PAS-positive reaction of the cytoplasm often were found in the periphery of the amyloid deposits.

The *liver* was involved in the amyloid deposition later than the spleen, but the deposition could always be produced by further treatment with cortisone or nitrogen mustard after discontinuance of the casein injections. In early stages the Kupffer cells in the sinusoids were swollen, hypertrophic, and contained pyroninophilic cytoplasm, whereas the picture in the early amyloid stage was dominated by large, hypertrophic Kupffer cells with distinctly PAS-positive cytoplasm in direct connection with the amyloid deposits situated between the sinus endothelium and the liver cells. The amyloid appeared in the liver as a product of secretion by the PAS-positive Kupffer cells of the sinusoids (Fig 3) in harmony with the typical localization.

The *kidneys* showed analogous cellular reactions to account for amyloid changes in the glomeruli. Stages characterized by proliferating pyroninophilic endothelial cells in the glomerular tufts were followed by the appearance of PAS-positive material in the same cells, showing direct transition to confluent amyloid masses in the loops.

Hyperimmunized Rabbits

Rabbits immunized with a culture of formaldehyde-killed Pfeiffer bacillus given in intravenous injections three times a week for periods varying from 7 to 16 months showed widespread granulomatous and necrotic lesions in the spleen and lungs and more scattered lesions in the kidneys, liver, adrenal glands, myocardium, and other organs. In addition, especially the spleen and lungs showed a very pronounced proliferation of mature and immature plasma cells. Local amyloid lesions of varying degree and different types of glomerular lesions in the kidneys often were visible.

Besides the marked aggregations of pyronine-positive plasma cells, especially in the red pulp, sections from the *spleen* showed numerous morphologically identical cells, characterized by a bright red nongranular cytoplasm in sections stained with the PAS method. Such PAS-positive reticulo-endothelial and plasma cells were found either in large accumulations (Fig. 4) or scattered in the pulp among the dense aggregations of pyronine-positive plasma cells; numerous Russell bodies showing similar cytochemical properties also were present in the neighborhood. The cytoplasm of proliferating reticulo-endothelial cells in the perifollicular zone and that of some reticulum cells of the germinal centers were also PAS-positive. A direct transition from PAS cells to amyloid deposition was evident in many areas.

The *lungs* were involved in all cases of this group; in addition to dense aggregations of pyroninophilic plasma cells they presented a broadening of alveolar septa showing proliferating septal cells with faintly PAS-positive cytoplasm and pronounced accumulations of large reticulum cells with eccentric nuclei and containing globular inclusions of finely granular, bright red PAS-positive material (Fig. 5). The PAS-positive material often was surrounded by a pale cytoplasmatic zone, which proved to be pyronine-positive in sections stained with the Unna-Pappenheim method. A few of these cells were of the giant cell type with several peripheral nuclei surrounding the PAS-positive material. Among the PAS cells several Russell bodies were seen also. These were often especially numerous around the vessels.

In local areas with closely packed PAS cells, a gradual transition to epithelioid cells was remarkable, and numerous cells with pronounced PAS-positive inclusions in the cytoplasm were observed regularly in the epithelioid cell granulomas. The findings suggested that PAS cells represented precursor stages directly involved in the genesis of amyloid, hyalin, and also of epithelioid cell granulomas and necrotic granulomatous lesions.

Similar cellular changes were found in the other organs. In the liver the proliferating Kupffer cells with strongly PAS-positive cytoplasm often showed a transition to local amyloid depositions.

The findings in sections from the kidneys stained with the PAS technique were of interest with regard to the histogenesis of certain glomerular lesions that occur in specific disease. In animals showing the picture of acute glomerulonephritis, the proliferation of endothelial cells of the glomerular tufts showed very marked pyroninophilia as stated in the previous study, and the glomeruli appeared bright red in contrast to the surrounding parenchyma. However, in sections

stained with the PAS technique, globules of a finely granular PAS-positive material were visible regularly in the proliferating cells (Fig. 6). Transition from swollen glomerular cells containing PAS-positive material in the cytoplasm to deposits of homogeneous material in the tufts, such as amyloid, was a striking finding and indicated a *cellular* origin locally of such lesions.

Administration of cortisone or ACTH induced a marked increase in homogeneous precipitates and a decrease in cells containing pyroninophilic or PAS-positive substance. In some cases of subchronic glomerulonephritis the various cellular stages could be found in the same section.

Guinea-Pigs Treated with Sodium Caseinate Injections

The changes in guinea-pigs were exactly similar to the findings in experimental amyloidosis in mice. In the treated guinea-pigs, the spleen also showed incipient amyloid changes with typical PAS cells in the border zone of the amyloid deposits.

Control sections from all three groups of experiments were exposed to the action of diastase (saliva), hyaluronidase, or ribonuclease. The PAS reaction was still positive after treatment, and the material was devoid of metachromatic properties.

The findings taken together suggest that the cytoplasmic material in the cells investigated consisted wholly or partly of mucoprotein or glycoprotein.

Discussion

The widespread proliferation of reticulo-endothelial and other cells derived from mesenchyme, characterized cytochemically by the presence of a PAS-positive material in the cytoplasm, suggests a most important function of reticulo-endothelial cells in producing polysaccharide-containing globulins (mucoproteins or glycoproteins) in response to diverse stimuli or injuries.

It is evident that the inverse relationship between ribonucleic acid and mucoprotein, not only in plasma cells forming Russell bodies, as demonstrated by Pearse[27] (1949), but also in reticulum cells, Kupffer cells of the hepatic sinusoids, endothelial cells of the renal glomerular tufts, and in endothelial and adventitial cells of vessels, supports synthesis and not absorption. As stated earlier, various noxious stimuli may produce a proliferation of reticulo-endothelial cells showing pyronine-positive cytoplasm. The present study shows that, following over-stimulation, a varying proportion of these cells will show a decreasing amount of pyronine-positive material (ribonucleic acid)

and an increasing content of PAS-positive substance (mucoprotein).

Mesenchymal derivatives, especially reticulum cells colored by the PAS technique, were directly involved in the genesis of a variety of morphologic lesions of mesenchymal tissue, such as the formation of amyloid and hyalin, and also linked with the morphogenesis of epithelioid cell granulomas and granulomatous necrosis. The rôle of PAS cells during the formation of amyloid is of interest, as it may throw light on the pathogenesis of this disorder. The occurrence of proliferating, hypertrophic, strongly PAS-positive reticulum cells in the spleen, liver, glomeruli, and vascular walls in direct relation to the also partly PAS-positive amyloid precipitates gives direct evidence of a local cellular secretion of polysaccharide-containing globulins, and is inconsistent with the concept of a precipitation of amyloid from the blood, caused by the raised serum glycoprotein or serum mucoid levels, as suggested by several workers. The findings may account for the chemical composition of amyloid, which is known to be a glycoprotein, and will also provide a reasonable explanation for the associated abnormalities in the serum protein-bound polysaccharides and the serum protein pattern during amyloid formation. These questions will be the subject of further study.

Obviously, the proliferation of PAS cells can be considered a general adaptation reaction, closely related to the pyroninophilic cellular reaction.

Summary

In response to antigenic and diverse unspecific stimuli (experimental amyloidosis, immunization), various types of mesenchymal cellular derivatives, especially reticulo-endothelial cells, widely dispersed in the organs, showed a marked content of globular or finely granular periodic acid-Schiff-positive substance in the cytoplasm. This was considered to be mucoprotein or glycoprotein produced by the cells.

PAS cells, pyroninophilic cells, and intermediate cell types containing PAS-positive as well as pyroninophilic material in the cytoplasm are considered characteristic functional stages of various types of mesenchymal (especially reticulo-endothelial) cells, including reticulum cells and cells of morphologic plasma cell type.

The current study shows evidence that mucoprotein- or glycoprotein-producing PAS-positive cells are directly concerned in the formation of amyloid, hyalin, and related substances in, e.g., the spleen, liver, and the renal glomeruli, and are involved in the genesis of epithelioid cell granulomas and various other lesions. The cytochemical findings suggest a local secretion of polysaccharide-containing globulins during the stage of amyloid precipitation.

Apart from accounting for the pre-eminent distribution of amyloid in the tissues rich in reticulo-endothelial components, the findings also provide a reasonable explanation for the associated abnormalities in the serum protein-bound polysaccharides (mucoproteins, glycoproteins), and indicate that reticulo-endothelial cells of PAS cell type are one of the chief sources of the production of serum mucoprotein or glycoprotein.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

All sections are from formalin-fixed tissue, stained by the periodic acid-Schiff technique of Hotchkiss.

FIG. 1. Stage of intensive amyloid formation in the spleen. Mouse 43 treated with injections of sodium caseinate for 30 days followed by ACTH injections for 4 days. PAS-positive reticulum cells in direct continuity with amyloid precipitates in the spleen. × 460.

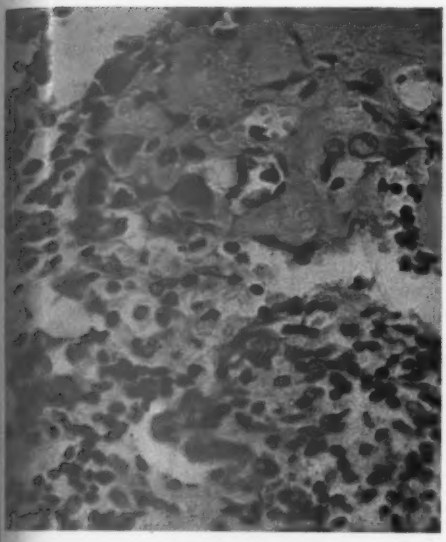
FIG. 2. Border zone of amyloid deposit in the spleen. Mouse 23 treated with injections of sodium caseinate for 30 days. Hypertrophic reticulum cells showing secretion of a homogeneous PAS-positive substance confluent with the amyloid precipitates. × 950.

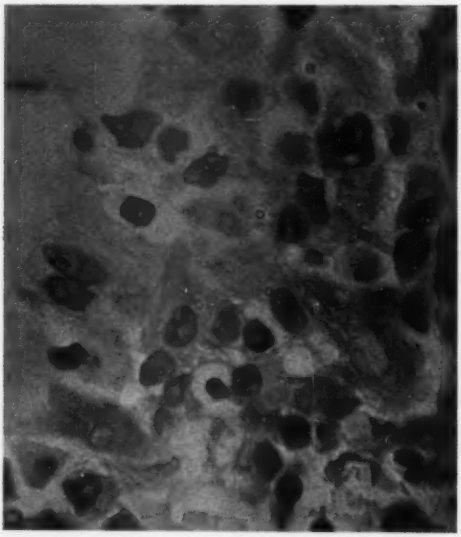
FIG. 3. Hypertrophic PAS-positive Kupffer cells of hepatic sinusoids in stage of amyloid formation. Mouse 23 treated with sodium caseinate injections and also with three injections of nitrogen mustard. × 460.

FIG. 4. Aggregations of PAS-positive plasma cells in the splenic pulp. Hyperimmunized rabbit 2966. × 460.

FIG. 5. Section from the lung showing collections of large reticulum cells with eccentric nuclei and containing globular inclusions of finely granular PAS-positive material. The surrounding pale cytoplasmatic zone was proved to be pyronine-positive in sections stained with pyronine-methyl green. Hyperimmunized rabbit 2927. × 950.

FIG. 6. Proliferating endothelial cells of the renal glomeruli, showing globules of a finely granular PAS-positive material. In sections from the same block stained with pyronine-methyl green the substance was found to be surrounded by pyronine-positive material which was removable by hydrolysis with ribonuclease. Hyperimmunized rabbit 3785. × 950.





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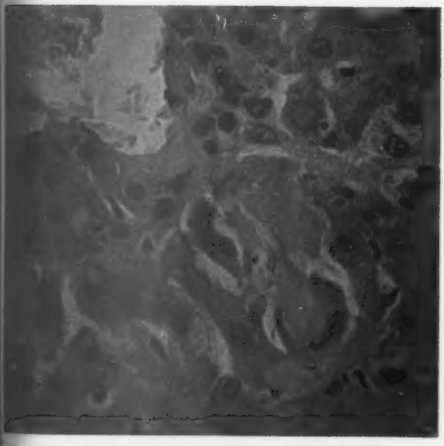

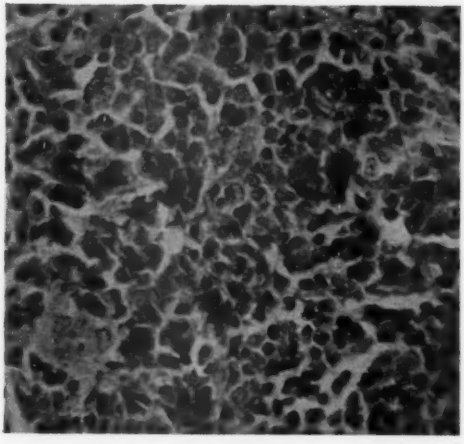

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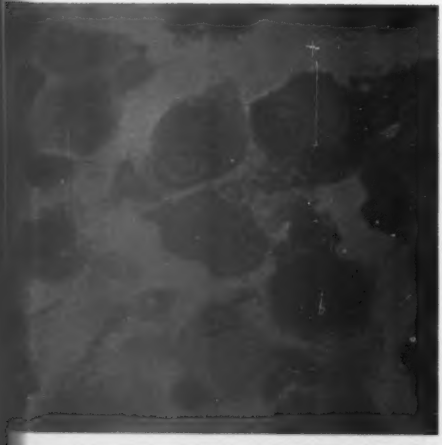

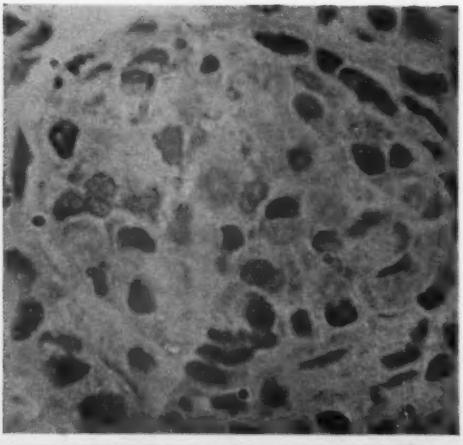

6

THE IDENTIFICATION OF NEOPLASTIC CELLS IN SEROUS EFFUSIONS

Critical Analysis of Smears from 2,029 Persons*

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The cytologic examination of sediments of serous exudates and effusates is of distinct value in confirming or disproving the presence of cells originating in malignant tumors metastatic to the cavities in which these effusions have formed. Only for mesothelial or synovial tumors could there be any hope of early detection of a primary growth and, since such tumors occur rarely and are seldom if ever recognized as mesothelial by the cytologist, the method is thus limited comparatively to the detection of cells from metastatic tumors. The determination of conditions other than neoplastic (such as cirrhosis or congestive heart failure) is very important, however, in averting unnecessary operative procedures; thus the method is of more value in connection with prognosis than it is with early diagnosis and prevention of further growth.

Methods of Cytologic Examination

There are two techniques for the demonstration of cells for cytologic examination: the conventional Papanicolaou smear and the "cell-block" method. In the former the sample of sediment is smeared over a glass slide and immediately fixed in alcohol and ether in equal proportions; in the latter procedure it is fixed in the centrifuge tube by any desired fixing fluid and forms a button of compacted, fixed cells which may then be removed and embedded in paraffin like any piece of tissue. The resulting block may be sectioned and stained as desired. In many laboratories a portion of the sediment is first removed and used for the preparation of smears while the remainder, if relatively undisturbed, is reserved for blocking. If it has been much disarranged by this manipulation, it may be necessary to repack the cells by further centrifugation.

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Comparison of the Two Methods

In a smear most of the cellular elements are spread out and discretely separated, while in the cell-block they lie closely apposed. Thus, the smear is preferable for detailed cytologic study and the cell-block for rapid evaluation of the cellular population of the sediment. The contrasting appearance of the resulting microscopic pictures is shown in Figures 11 and 13 to 16. While cell-blocks have much to offer and should be employed as a routine procedure, practically all of the diagnoses concerned in the present investigation were made on smears, as it is the policy in the Papanicolaou Laboratory to rely solely upon such examination and cell-blocks are not prepared. From past experience, however, it seems to be generally advisable to use both techniques and to check the results of the examination of one against those of the other. Cell-blocks are particularly valuable in ruling out non-neoplastic conditions like hepatic cirrhosis and congestive heart failure as causative factors in the production of the cells observed. Comparison of each cell with its fellows is all-important and when the cells are closely packed in an optical field, this is more readily accomplished than when they are widely dispersed in the smear. Having thus stated the case for the cell-block, which I have found to be practical and reliable in earlier investigations[1] and therefore wish to commend, it will not be discussed further in this paper, which deals with results obtained wholly from smears.

Recognition of Neoplastic Cells

There is little that is really difficult about recognizing cells from malignant tumors in smears, provided that they are abundant and characteristically abnormal. The classic criteria of malignant change in cells are too well known to require restatement here. The presence in smears of fragments of tumor or of clusters of neoplastic cells is very helpful and may be decisive in arriving at a diagnosis. While non-neoplastic cells also may form clusters, they tend to be closely apposed within the group and not loosely applied and somewhat set off or spaced as are those of neoplastic origin. This feature has been noted and stressed by Koss.[2] When, however, the cells are few and ungrouped, the normal mesothelial lining elements can be very misleading, particularly so if they have undergone metaplasia in response to inflammatory stimuli. These cells and the histiocytes require careful study and familiarity so as to provide the cytologist with normal

standards before he attempts the diagnosis of neoplastic elements. A recent and excellent article by Luse and Reagan[3] will be found to be very helpful in supplementing such a study. Their material represented the examination of sediments from fluids from some 1,000 patients; I have just completed the investigation of smears from 2,029 consecutive cases, taken from the files in our laboratory, and have found that the results of this review compare favorably with theirs.

Report on the Investigation of 2,029 Specimens

The 2,029 cases investigated furnished more than five times that number of smears, each of which was studied with sufficient care to afford definite conclusions as to the presence or absence of cancer. They had all been screened by the laboratory staff, suggestive fields had been marked with ink-dots, and they had been reviewed and reported upon by senior cytologists. While the staff had cognizance of the clinical data on the cases concerned, mostly well documented, I preferred to examine the smears without knowledge of the histories of the patients, believing that subjective reasoning and bias might be eliminated when nothing was known except the serial number of the specimen. Examination of the smears was focussed largely upon the marked fields and entire smears were seldom scanned systematically as were those reported upon in a previous article[4] dealing with the identification of the site of origin and type of tumor.

Sources of the Fluids Examined. The material comprised specimens of sediments of fluids from the pleural (1,301), peritoneal (700), pericardial (28), and articular cavities (17); three taken from hydrocele sacs made up the total of 2,049 which, as it will be seen, exceeds that of the cases examined by 20. This is explained on the basis that specimens of more than one type of fluid (e.g., pleural *and* peritoneal) sometimes were sent in from the same patient. Most of the sets of smears represented a single submittal from a single cavity, but several of them comprised a series of taps made at intervals on several successive dates, often four or more. All of these were examined and compared; oftentimes cells that were noted and suspected in early specimens disappeared from later ones, demonstrating the advisability of submitting several successively obtained specimens whenever possible. It was the pleural and peritoneal fluids that were of chief interest in this investigation; only one of the pericardial specimens showed cells from a malignant tumor while those from

joints and hydrocele sacs were all negative. Table I shows the distribution of the specimens.

Specimens Positive for Cancer. There were 610 positive diagnoses, or 30.1 per cent of the specimens. As will be explained later, a subsequent revision of the false positive diagnoses among these, made

TABLE I
Types of Fluids Examined

	Pleural	Abdominal	Pericardial	Synovial	Hydrocele	Total
Number	1,301	700	28	17	3	2,049
Per cent	63.5	34.2	1.4	0.8	0.1	100

in the light of knowledge of the source of the fluid, the clinical history, the sex and age of the patients concerned, and experience accumulated during the course of the examination, cut this total from 610 to 583 (30.1 to 28.8 per cent). Positive cases were rated Class IV or V.

Specimens Doubtfully Positive for Cancer. Doubtfully positive (Class III) diagnoses totalled 451, or 22.2 per cent. This rather high figure may be attributed to ignorance of clinical data, which would conduce to doubt and some skepticism in grading the smears. For example, if it were known that a given specimen was peritoneal fluid from a man of 50 years who was suffering from chronic alcoholism and suspected of having hepatic cirrhosis, the diagnosis of possible carcinoma, while not excluded, would at least be more unlikely after the smears had been examined and analyzed. The revision of false positive diagnoses, already referred to, did not affect the total of Class III reports.

Specimens Negative for Cancer. Negative diagnoses totalled 968, or 47.7 per cent. After revising the false positive diagnoses, this figure was increased to 995, or 49.0 per cent.

Table II presents the distribution of these diagnoses.

TABLE II
Distribution of Diagnoses in Cases Examined

	Positive	Doubtful	Negative	Total diagnoses
Number	610	451	968	2,029
Per cent	30.1	22.2	47.7	100
After revising false positive diagnoses				
Number	583	451	995	2,029
Per cent	28.8	22.2	49.0	100

REMARKS ON POSITIVE DIAGNOSES

Validity of Positive Diagnoses

Of the 610 positive diagnoses, 434, or 71.1 per cent, were subsequently confirmed by reliable data such as necropsy, biopsy, or incontrovertible clinical evidence. Ninety-four, or 15.4 per cent, were unconfirmed. These 94 patients were found to be chiefly in the private practice of physicians or from hospitals beyond our range of ready "follow-up." The bulk of the material, however, came from New York, Memorial, or Bellevue Hospitals and was reliably documented. As the matter of false positive diagnoses is the most significant and important part of this report, it will now be discussed at length.

Special Consideration of False Positive Diagnoses

The decision as to whether a given diagnosis was true or false rested upon the final discharge diagnosis of the hospital involved in the care of these patients; this was the only practical way in which the reports could be standardized. Such discharge diagnoses were almost always based upon reliable data, as defined; there was a small minority (about 10 per cent) in which the discharge diagnosis was open to question. Where the cytologic findings seemed to be strongly at variance with these diagnoses, there was room for doubt; the opinion of the cytologists may have been right and the general impressions of the clinicians wrong.

After determining the false positive reports in this way, a careful review of the smears involved was carried out in an attempt to learn the reason for the errors. If, after reviewing the data on the case together with the smears, a patient's smears no longer appeared to suggest the presence of tumor, that case was transferred from the positive to the negative column and appropriate adjustment was made in the totals. Even after this re-evaluation there were several instances in which the original (false) positive findings could not be altered with a clear conscience—the smears appeared to be just as menacing on review as they had been originally. In such cases it is possible that the discharge diagnoses were, in reality, "false negatives." For example: A patient was diagnosed as having a pleural effusion positive for cancer, but necropsy determined that death was due to pulmonary tuberculosis with pleuritis. It may seem presumptuous to suggest that a small bronchogenic carcinoma may have coexisted with tuberculosis and, because of its diminutive size, may have been undiscovered in a lung that was riddled with tuberculous lesions. Such difficulties have developed on occasion.[5] This review revealed that

hepatic cirrhosis, congestive heart failure, and tuberculosis were the conditions most frequently misdiagnosed as malignant tumor by the cytologist.

Cirrhosis. Seventeen mistakes were made in diagnosing cirrhosis; after review, 13 smears were judged to be negative, but the other 4 still appeared to warrant a positive diagnosis of malignant tumor; were these 4 to be resubmitted as presumably new specimens, they would still be considered positive. Figures 1, 3, 11, and 13 illustrate the pitfalls that exist in diagnosing this condition. How can these be overcome? It was found that the mesothelial cells in the smears were almost always to blame for such mistakes. Histiocytes sometimes were troublesome also. Both of these cell-types can be recognized by their uniformity in shape and size (though this may vary in histiocytes) and by their low "n/N ratio" (obtained by dividing the diameter of the nucleolus by that of the nucleus, as proposed by Quensel[6,7]). I found this procedure to be helpful when making a similar investigation in 1937.[1] The n/N ratio of non-neoplastic cells usually lies between 0.15 and 0.20, while that of neoplastic cells ranges between 0.25 and 0.40. After acquiring experience while making these measurements, it becomes possible to estimate the ratio with sufficient accuracy to establish the diagnosis without resorting to the ocular micrometer.

Cells in cirrhotic ascitic fluids show low n/N ratios; they tend to be grouped in tightly integrated clusters which may be ovoid, or may have a close resemblance to epithelial pearls. They may even produce rosette-like groups or pseudo-acini, as has been shown in the article of Luse and Reagan.[3] They are illustrated in Figures 1, 3, and 11 of the present article. They often are found in mitotic division, but the figures are normal and delicate, rather than coarse and disorderly. Under the stimulation of inflammation, the karyosomes may become misleadingly coarse and overstained, while the cytoplasm may show extensive vacuolization and resemble that of the signet ring cells of mucous carcinoma. Mesothelial cells can be avidly phagocytic, particularly in the presence of pus.

Histiocytes may become large and comparatively dense, and their nuclei may undergo some metaplastic thickening and hyperchromasia.

Congestive Heart Failure. Congestive heart failure produces similar fluid sediments and hence there is confusion similar to that caused by cirrhosis. There were 13 false positive diagnoses in this group which, after revision, were reduced to 5. The cells presented in slightly smaller numbers than they did in the ascitic fluids, a fact that

increased the difficulty in diagnosis. Here again it is a matter of carefully studying the nuclear characteristics and paying due attention to the n/N ratio.

Tuberculous Inflammation. There were 12 false positive diagnoses on fluids which proved to be tuberculous rather than neoplastic in origin. Revision reduced this number to 6. It should be emphasized that lymphocytes are often very numerous in tuberculous exudates, leading to a misdiagnosis of lymphoma. The mesothelial cells are dense and substantial and the histiocytes take on their familiar epithelioid appearance, all of which makes for difficulty in decision. Langhans giant cells are present to be sure, Luse and Reagan[3] having found them in 11 per cent of their tuberculous fluids, but as they noted them also in 32 per cent of cirrhotic effusates and in 31 per cent of those resulting from congestive heart failure, such giant cells cannot be said to offer much assistance in diagnosis.

Lupus Erythematosus Disseminatus. There were 3 cases of disseminated lupus in this series, one of them falsely diagnosed as malignant tumor. A study of the smears revealed large numbers of cells that appeared more histiocytic than mesothelial ("L. E. cells"). They had a rather denser cytoplasm than did histiocytes and their nuclei were irregularly elongated, lobulated, or multiple and somewhat resembled those of Reed-Sternberg cells. They were seldom conspicuously vacuolated.

Negative Cases Contrasted with False Positives

Were we to drop the topic of false positive diagnosis at this point, a misleading impression might be created. The question arises: How many of these borderline and difficult diagnoses were correctly made in the course of the study? Thus far we have been concentrating on errors; how about the successful diagnoses? Tables III and IV present the results obtained in connection with false positives (Table III) and the revision of these combined with a tabulation of correct diagnoses of the troublesome conditions just discussed (Table IV). The former table is self-explanatory; in Table IV the conditions are listed in the left-hand column, next come the total number of cases studied, and then the number and percentage of those correctly diagnosed. The column labelled UFP lists the number of unrevised false positives and is followed by their percentage of total cases. The next column presents the revised false positive diagnoses (RFP) by numbers and is followed by percentages of totals. Finally, the changes

brought about by the revision are set down by number and percentage of total cases.

Of 113 cirrhotic ascitic fluids, 96 were correctly diagnosed as non-neoplastic, or 85 per cent; the other conditions showed less accuracy in diagnoses and fell in the 60 to 65 per cent range. Study and revision, however, were fruitful and there was a 20 per cent reduc-

TABLE III

Comparison of Types of Positive Diagnoses

	Confirmed	Unconfirmed	False	Total diagnoses
Number	434	94	82	610
Per cent	71.1	15.4	13.5	100
After revising false positive diagnoses				
Number	434	94	55	583
Per cent	74.4	16.1	9.5	100

TABLE IV

Analysis of Correct Negatives and False Positives

	Total	CN	% Tot.	UFP	% Tot.	RFP	% Tot.	Ch.	% Tot.
Cirrhosis	113	96	85.0	17	15.0	3	2.6	14	12.5
Congestive heart failure	34	21	62.0	13	38.2	5	14.7	8	23.5
Tuberculous inflammation	31	19	61.0	12	39.0	6	19.4	6	20.0
Pulmonary infarct	13	8	61.5	5	38.5	5	38.5	0	0.0
Pleural effusion	5	4	80.0	1	20.0	1	20.0	1	20.0
Lupus erythematosus disseminatus	3	2	66.6	1	33.4	1	33.4	0	0.0
*Pneumonia	2	2	100.0	0	0.0	0	0.0	0	0.0
Totals	201	152	75.6	49	24.4	21	10.4	29	14.4

* Pneumonia included in this list because of mistaken diagnosis on two cases in laboratory reports. CN=correct negatives; % Tot.=percentage of total given in column 1; UFP = unrevised false positives; RFP = revised false positives; Ch. = change.

tion in false positives in congestive heart failure and tuberculous inflammation. Pulmonary infarct, however, was unchanged by the revision. It remains a very troublesome condition as it produces highly metaplastic mesothelial cells. It should be pointed out that the false positive diagnoses, when reckoned in percentages, apply only to the conditions listed in the table and not to the series as a whole, in which they were far lower.

The results just discussed apply only to the diagnoses on a selected

series of troublesome, non-neoplastic conditions. When we consider the whole series of 2,029 cases, there are 13.5 per cent false positive diagnoses before the revision and 9.5 per cent after it. This shows improvement over the figures obtained during an examination of cell-blocks in 1937[1] in which correct positive diagnoses ranged from 65 to 70 per cent, according to the nature of the sediments examined.

REMARKS ON DOUBTFULLY POSITIVE DIAGNOSES

All Class III diagnoses were reviewed and compared in order to ascertain how many of the patients whose fluids were thus graded actually had cancer and how many did not. Clinical data on these patients were difficult to obtain, as the staff was more apt to collect and to check data with definitely positive or negative, rather than doubtfully positive diagnoses. Of the 451 Class III diagnoses, 135 were found to apply to patients who had proved carcinoma (30.0 per cent). There were 238 diagnoses that could not be effectively documented and on patients proved to be non-cancerous there were 100, or 22.2 per cent.

DEGREE OF AGREEMENT IN DIAGNOSIS WITH LABORATORY STAFF

It would be quite natural for the reader to be curious as to how my diagnoses compared with those of the laboratory staff, which comprised several screeners and cytologists, the make-up of the personnel varying over the decade covered by the investigation. Reference to Table V will show that the degree of agreement has been

TABLE V

Degree of Agreement in Diagnosis with Laboratory Staff

	Complete agreement	Technical disagreement	Essential agreement		Definite disagreement	Total diagnoses
	A	B	C		D	
Number	1,368	503	1,871	(A+B)	158	2,029
Per cent	67.4	24.8	(92.2)		7.8	100

entered in four columns under the headings complete agreement, technical disagreement, essential agreement, and definite disagreement. Two of these terms need no elucidation. Technical disagreement is an expression that implies a difference in the exact class allotted to the final diagnosis without indicating serious divergence in meaning. If a fluid graded Class II by the staff were to be rated Class III by me, this would be a technical disagreement since a rather

fine shade of interpretation may have swung the balance one way or the other. In the first instance the staff, unwilling to suspect the presence of tumor on the basis of their evidence, issues a Class II diagnosis; in the second, I am unwilling to overlook certain dubious aspects of the smears and, disinclined to concede that the presence of tumor has been entirely ruled out, issue a Class III diagnosis. In contrast to this, a Class II diagnosis that implies the absence of cancer is so widely at variance with one of Class IV, which indicates good evidence of its presence, that the divergence of opinion should be listed as definite disagreement.

Complete agreement was attained in 1,368 (67.4 per cent) of the diagnoses before the revision; technical disagreement was noted in 503 (24.8 per cent) and definite disagreement in 158 (7.8 per cent) of the series. If it be permissible to combine the columns of complete agreement and technical disagreement under the heading of column C, which is essential agreement, there would be a 92 per cent agreement in diagnosis between the staff and me, which is excellent. Since I worked in ignorance of any data except the serial number of the smear, this is very close agreement and the figures indicate the comparative values of examinations that are purely objective and those in which subjective data are adduced in making the diagnosis.

RESULTS OF RAPID DIAGNOSIS OF TYPE AND SITE OF ORIGIN OF TUMORS

In view of the fact that I published an article on the rapid diagnosis of type and site of origin of tumors 2 years ago,[4] it was of interest to ascertain how accurate a determination of the types and original sites in the present series could be made in a cursory fashion while examining a far larger number of smears with the primary purpose of diagnosing the presence of tumor. In the former investigation only Class IV smears were examined; they were very carefully studied, considerable time being devoted to each smear. In the present work all fluids (irrespective of their grades) were examined rapidly to determine the presence or absence of tumor. The results in this examination were so disappointing that it became evident that such determinations can be carried out with reasonable accuracy only when considerable time and study can be devoted to each smear. Diagnoses of the probable type and site of origin of all tumors observed in the course of this present investigation were listed and analyzed, but the final results were too mediocre to warrant further discussion or publication.

Summary

The examination of cells in serous effusions is of distinct value in confirming or ruling out suspicions of tumor. It does not assist in the early detection of malignant growths, since these are already far advanced when cells are exfoliated into these effusions. Mesothelial cells and histiocytes present the chief stumbling-blocks in the interpretation of smears or sections of cell-blocks, as they may be confused readily with cells shed from malignant tumors after they have undergone metaplasia attributable to inflammatory, rather than neoplastic stimuli. The determination of the types of tumors found in smears of these fluids as well as their probable site of origin is possible only after special and rather prolonged study of each smear. Cursory examinations such as suffice for the assignment of a class or grade to a smear will not suffice to establish the more subtle features just mentioned.

So far as accuracy in diagnosing the presence or absence of malignant tumor in smears of serous fluids is concerned, of 610 positive diagnoses 434, or 71.1 per cent, were confirmed by reliable data, such as necropsy, biopsy, or incontrovertible clinical evidence. Among the 610 positive diagnoses, there were 82 that were proved to be false; revision of these diagnoses, made with the aid of further study and reference to the clinical data which were unknown during the original examination, reduced the total to 55, or 9.5 per cent, which is not excessive. This revision also reduced the total of positive diagnoses from 610 to 583, or 28.7 per cent of the total series compared to the original 30.1 per cent. There were 968 negative reports, which were increased to 995 by this revision; or 47.7 per cent increased to 49.0 per cent. The comparatively large percentage of positive reports in this series is attributable to the fact that the fluids sent in for appraisal came from patients suspected of harboring malignant tumors, rather than from those merely plagued with effusions. A comparison of my diagnoses with those of the members of the staff demonstrates a very satisfactory degree of agreement. Complete agreement existed in 67.4 per cent of the diagnoses, essential agreement in 92.2 per cent; this is discussed fully in the text of this paper.

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LEGENDS FOR FIGURES

All photomicrographs were taken by Mr. Constantine Railey at a magnification of × 600.

FIG. 1. Cluster of mesothelial cells from sediment of ascitic fluid in cirrhosis. Double contour suggested.

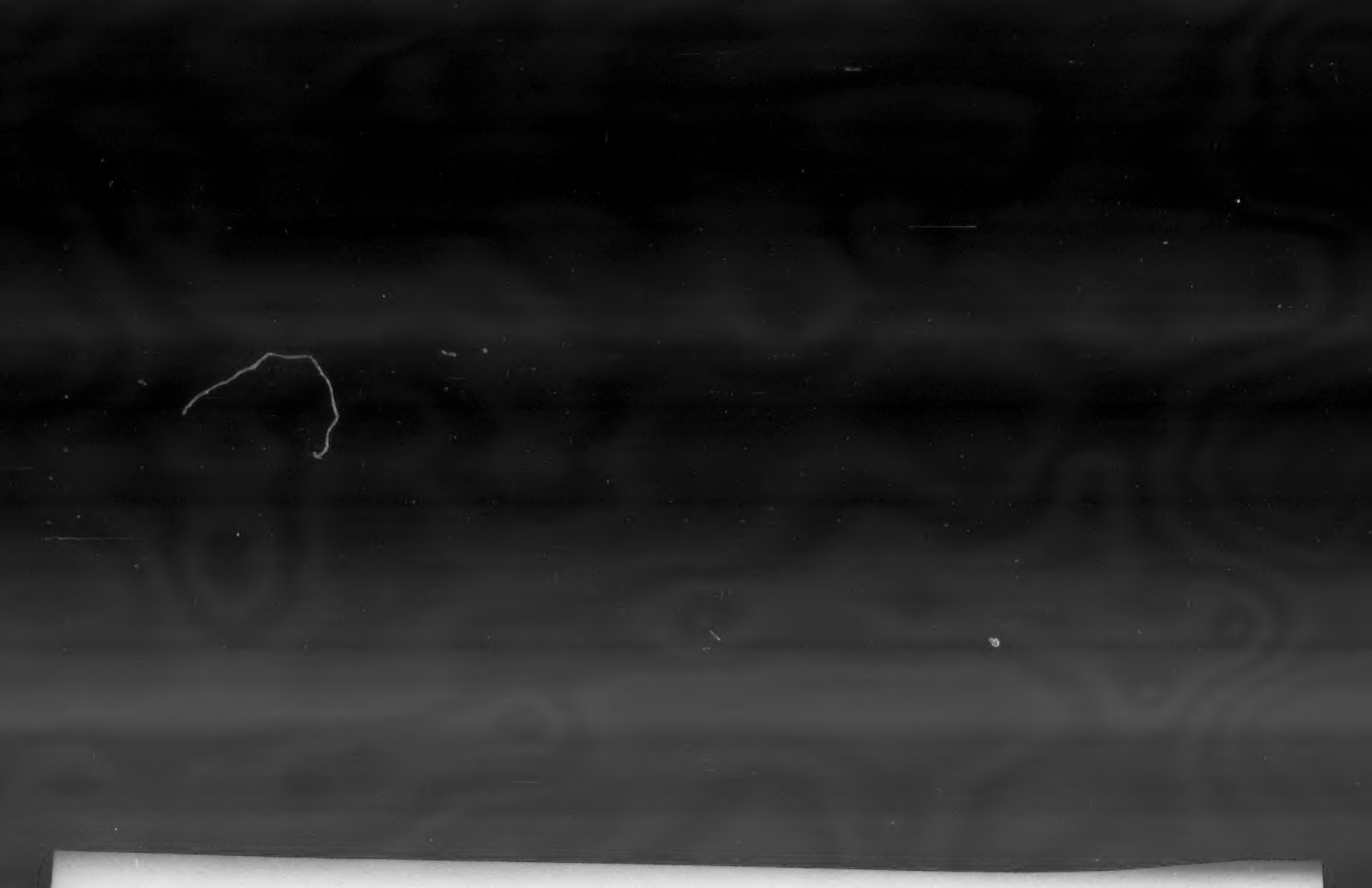
FIG. 2. Concentrically arranged cluster of mesothelial cells in which double contour is clearly evident.

FIG. 3. Mesothelial cells from ascitic fluid in a case of cirrhosis; both laboratory staff and I were misled into a false positive diagnosis by the metaplasia present in these cells.

FIG. 4. Metaplastic mesothelial cells from a fluid from a patient with tuberculous pleuritis. Double contour may be noted.

FIG. 5. Multinucleated and highly metaplastic cell from a fluid of undiagnosed causation. Discharge diagnosis: "Amyloidosis of skin and kidneys." Laboratory staff and I both classified this as Class V.

FIG. 6. Extraordinary cells, from sediment of pleural effusion, diagnosed by both laboratory staff and myself as Class V. Discharge note: "No tumor demonstrated at thoracotomy." This does not disprove that a tumor might have been overlooked.



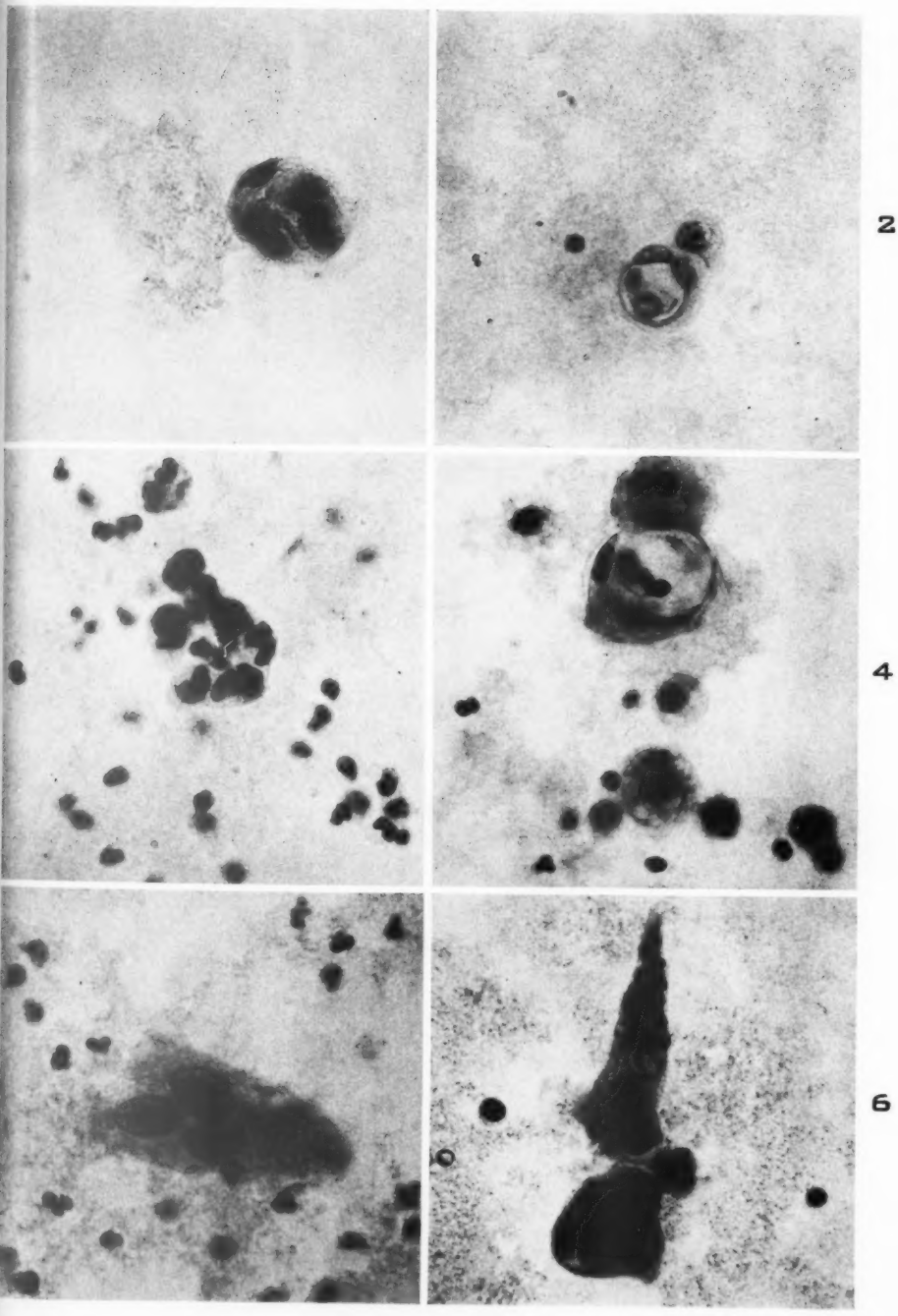


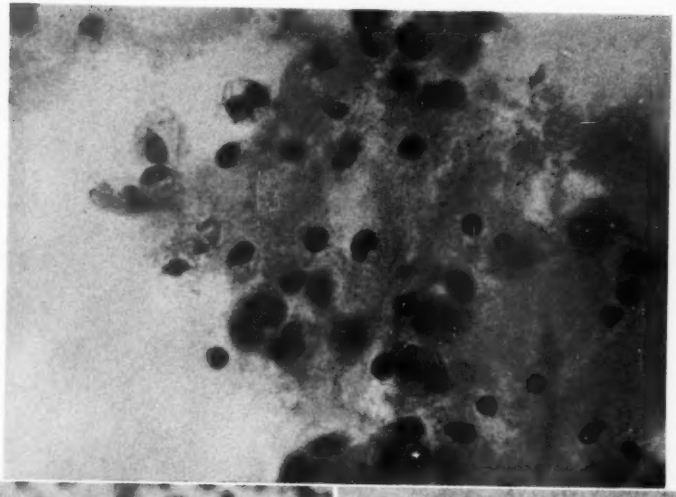
FIG. 7. Histiocytes in a pleural effusion, diagnosed as Class I.

FIG. 8. Metaplastic mesothelial (?) cells from exudate in empyema. Acute inflammation often causes this metaplasia and resulting confusion in diagnosis.

FIG. 9. Histiocytes from a peritoneal effusion, diagnosed as Class I.

FIG. 10. Concentric cluster of probable histiocytes; the cytoplasm is clear and transparent, the cellular outline is indistinct. Not suggestive of neoplastic origin.

FIG. 11. Two vacuolated mesothelial cells and some histiocytes from a smear of sediment of ascitic fluid in cirrhosis.



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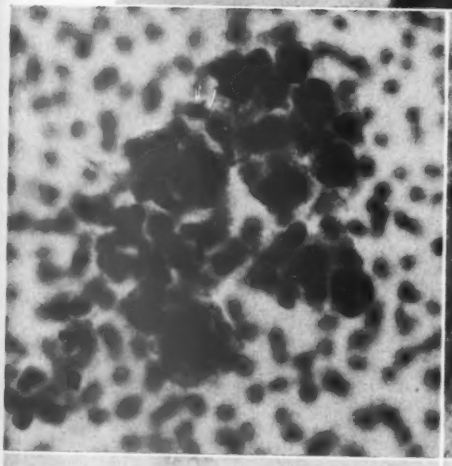

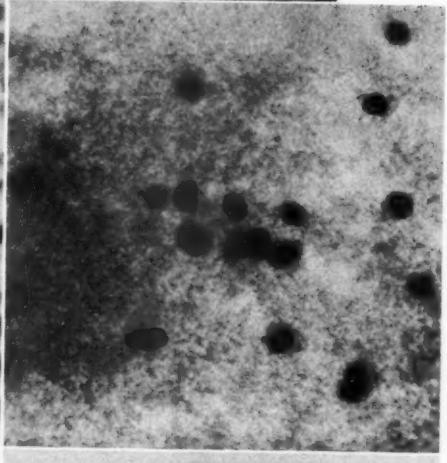

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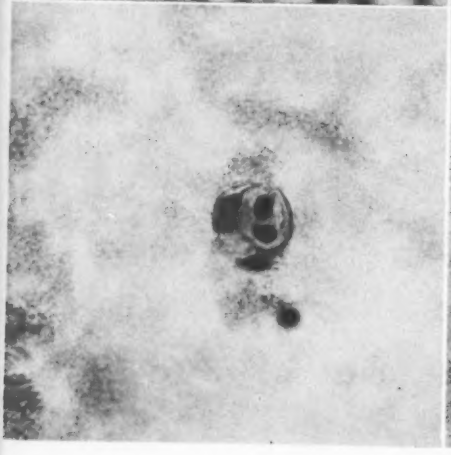

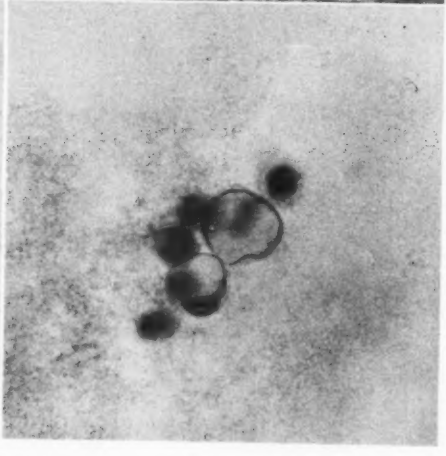

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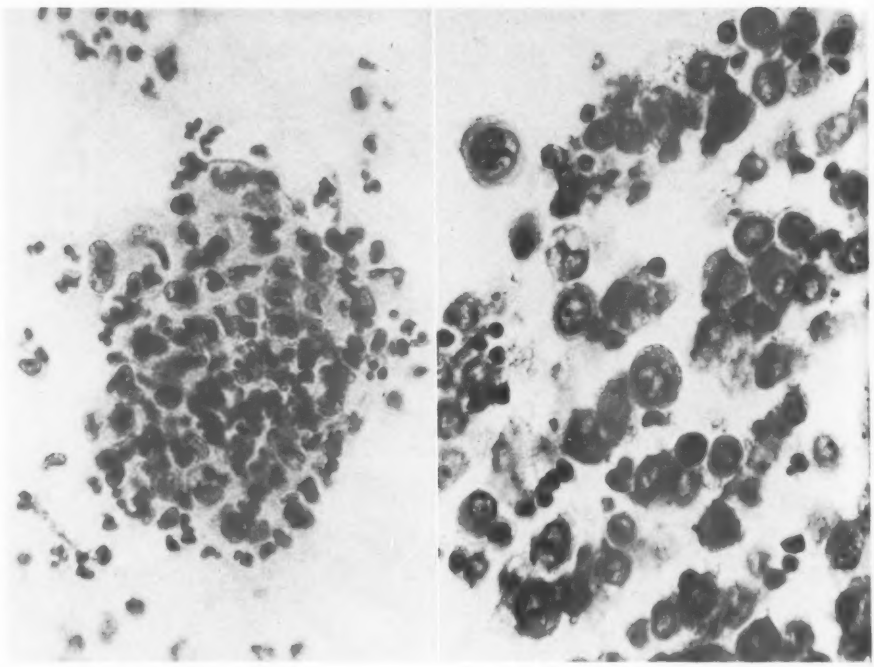

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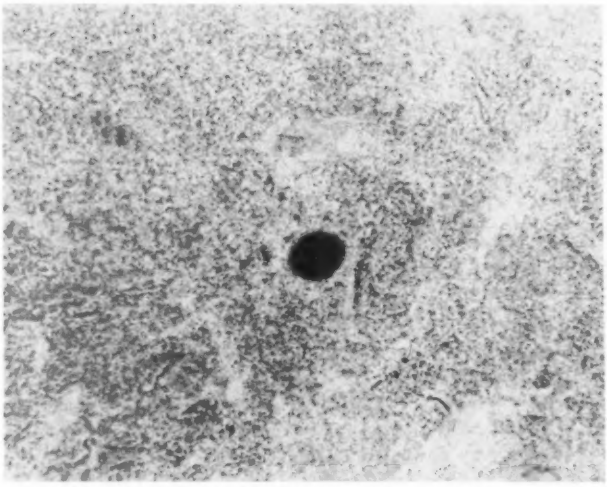
FIG. 12. Large cluster of probable "L.E. cells" from pleural effusion in lupus erythematosus disseminatus. Nucleoli are small and not prominent.

FIG. 13. Section of a cell-block prepared from the same fluid as that shown in Figure 11, for comparison with that figure. While metaplastic, these cells show small nucleoli and a low nucleolar-nuclear ratio. (See text.)

FIG. 14. Completely isolated cell from an ovarian carcinoma in smear of sediment from a pleural effusion.

FIG. 15. Field at periphery of section from cell-block made from the same sediment. Although neoplastic cells are still sparsely represented, several of them are congregated in this microscopic field, rather than just one.

FIG. 16. Section from a cell-block of sediment of fluid from a case of pseudomucinous adenocarcinoma of ovary. There is a rosette-like arrangement of the neoplastic cells. Unfortunately this characteristic picture is not regularly present in cases of ovarian carcinoma. (Cf. Fig. 15.)



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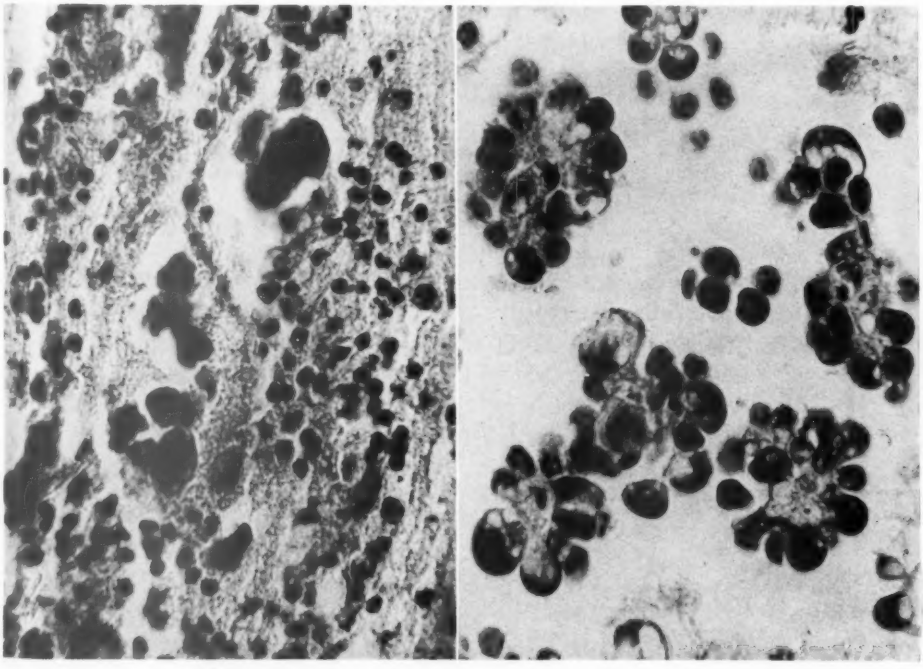

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EFFECT OF CARTILAGE AND OTHER TISSUE SUSPENSIONS ON REPARATIVE PROCESSES OF CORTISONE-TREATED ANIMALS*

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In previous reports from this laboratory,[1,2] experimental evidence has been brought forth which suggests that cortisone in adequate doses probably blocks the release from injured tissues of chemical substances which are necessary for the series of events known as inflammation and repair.

Experiments aimed at identifying this elusive material have continued. Hyaluronic acid, hyaluronidases (from testicular and bacterial sources), lysozyme, ion exchange resins, embryo extract, gelatin, oxidized cellulose, collagenase, suspensions of talc powder, and mechanical trauma have failed in successive series of cortisone-treated and control rats, using the method described in a previous paper,[2] to overcome appreciably the effect of this steroid (unpublished experiments).

In a more recent series of experiments, suspensions and homogenates of different tissues were tested for their possible rôle in modifying the cortisone-induced depression of the reparative process.

METHOD, MATERIALS, AND RESULTS

A. Cartilage

It has been our suspicion that the substances which fail to be released in the injured tissues under the effect of cortisone are probably mucopolysaccharide in nature.[1,2] Consequently, suspensions of powdered cartilage of different origins, which are rich sources of acid mucopolysaccharides and especially of the chondroitin sulfates, were tested.

These studies were made on 29 cortisone-treated and 21 control rats. White male Wistar rats weighing around 200 gm. were given a daily intramuscular injection of 15 mg. of cortisone acetate until sacrificed. On the fourth day, a sterile surgical gauze pledget was

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† Fellow of the Canadian Arthritis and Rheumatism Society.

placed aseptically in the back at the termination of a subcutaneous tunnel, using a technique described previously.[3]

The following preparations were used. 1. Commercial powdered bovine tracheal cartilage prepared by short treatment with acid pepsin was autoclaved for 45 minutes in steam and a suspension was made in the proportion of 1 gm. to 7.5 ml. of an antibiotic solution, each ml. containing 4,000 units of penicillin and 8 mg. of streptomycin. Ten cortisone-treated and 6 control rats were used in this experiment.

2. Finely ground bovine tracheal cartilage, not treated with acid pepsin, was autoclaved and then added to the antibiotic solution, as outlined previously. Ten cortisone-treated and 3 control rats were used in this experiment.

3. Fresh pig's knuckle cartilage was finely minced and put in the antibiotic solution in the same proportions. Four cortisone-treated and 4 control rats were used in this experiment.

4. Finely ground bovine tracheal cartilage was subjected to extraction with water and the insoluble residue was used. Five cortisone-treated and 5 control rats were used in this experiment.

Each day 1 ml. of one of the cartilage preparations and 1 ml. of penicillin-streptomycin mixture were injected through the skin onto the pledget. Control groups received cholesterol suspension in the same vehicle, intramuscularly, in lieu of cortisone acetate. On the fifth postoperative day the animals were sacrificed and bacterial cultures were taken from the gauze pledgets. Infection, or at least positive cultures at necropsy, occurred in 5 cortisone-treated and 4 control animals. These were not included in the analysis of the results since it had previously been demonstrated that pyogenic infection might overcome the inhibitory effect of cortisone on granulation tissue.[2]

Results. In contrast to the picture previously reported[1,2,4] in which the pledgets remained free in cortisone-treated animals, the pledgets were, in general, encapsulated and adherent to the surrounding tissue of the cortisone-treated, cartilage-injected animals and some force had to be employed to free them. The microscopic examination (Fig. 1) showed consistently abundant granulation tissue, including large numbers of fibroblasts and new blood vessels, with minimal acute inflammation. This effect was purely local and dependent upon contact with the cartilage, since the skin wounds of the cartilage-injected, cortisone-treated animals showed little or no connective tissue repair. In these experiments the acid-pepsin-treated, autoclaved, beef tracheal cartilage apparently was more potent in producing this picture than

either autoclaved beef tracheal cartilage or fresh pig's knuckle cartilage. These preparations, obviously, cannot be compared quantitatively because crude suspensions were utilized and consequently there were variations in the amount of cartilage in each milliliter of suspension. The amount of suspension in contact with the pledgets also varied from day to day and from animal to animal and two of the preparations were autoclaved, while the fresh cartilage was not dehydrated.

In the cholesterol-treated control rats, reparative functions were very abundant and the pledgets were encased in a firm capsule of granulation tissue (Fig. 2).

B. Beef Bone

Experiments similar to those of group A were made on 12 cortisone-treated and 3 control rats. The same technique was used, only instead of cartilage a suspension of acetone-dried, powdered, but not decalcified beef bone was used in 6 cortisone-treated animals and in 3 controls; and bone spicules from human ilium were used in 6 cortisone-treated animals. The material was inserted with the pledget at the time of operation but not injected daily, as in the previous experiments. When the animals were killed on the fifth postoperative day, the gauze pledgets in the cortisone-treated rats were either free or loosely adherent. Microscopically, minimal fibro-angioplasia was present in the tissues surrounding the gauze. In the control animals the gauze pledget was encapsulated (Figs. 3 and 4) and there was abundant granulation tissue surrounding it.

C. Beef Tendon

Using the same technique, autoclaved, shredded beef tendon suspension was used daily in 13 cortisone-treated and 8 control rats. At necropsy on the fifth day the gauze pledgets of the cortisone-treated animals appeared to be surrounded by a semitransparent adherent membrane; and, microscopically, focal areas of loosely cellular, young granulation tissue were seen (Fig. 5). In the controls, there was the usual firm encapsulation consisting of abundant granulation tissue (Fig. 6).

D. Chondroitin Sulfate C

Four cortisone-treated and 4 control rats were used in a similar experiment, in which chondroitin sulfate C (dose: 1 cc. of a 2 per cent solution) was injected daily upon the implanted pledget of gauze.

On the fifth day, necropsy showed that the foreign body was either free or loosely attached to the surrounding tissues. Microscopic study showed rare and small foci of minimal fibro-angioblastic proliferation (Fig. 7). The controls showed the usual fibrous granulation tissue response (Fig. 8).

E. Chondroitin Sulfate A and Gelatin

Ten cortisone-treated rats, prepared as before, received daily injections of chondroitin sulfate A and gelatin (6 gm. of chondroitin sulfate and 2 gm. of gelatin in 75 cc. of distilled water). One cc. was injected daily upon the implanted foreign body. No controls were used. On the fifth day, necropsy showed that the gauze pledget was moderately adherent to the surrounding tissues by a thin hyperemic membrane. Histologically, minimal to slight focal repair (Fig. 9) was observed, the foci of granulation tissue being associated with pools of mucoid metachromatic material.

F. Umbilical Cord

In 6 cortisone-treated rats, using the same technique, dried (human) umbilical cord was introduced subcutaneously together with the pledget of gauze. No controls were used in this experiment. On the fifth day, necropsy showed minimal to moderate reparative tissue reaction around the foreign body (Fig. 10).

In all of these experiments, the skin wounds of the cortisone-treated animals, which were not in immediate contact with the substances tested, failed to show evidence of repair.

Discussion

From previous studies[1,2,4] it has been suggested that under the influence of cortisone, some substance, perhaps chemotactic for the cells necessary to complete the reparative process, is not elaborated or is inactivated locally. The observation that a suspension of autoclaved cartilage can overcome the cortisone effect seems to imply that this postulated substance is present in such a suspension and is not heat labile because it withstands steam sterilization. The fact that minimal to moderate reparative responses were obtained using suspensions of bone, tendon, and umbilical cord, respectively, should not be surprising in the light of our working hypothesis. In fact, the composition of the ground substance of these tissues is, in certain respects, similar to that of cartilage, even though it differs in concentration and patterns of mucopolysaccharides.

The practically negative results obtained with daily injections of chondroitin sulfate C solutions might be due simply to the rapid disappearance of a water-soluble substance from the site of injection. The more marked effect of chondroitin sulfate A and gelatin might result from less rapid diffusion due to binding by the gelatin. Of course, there may be other reasons. For instance, these acid mucopolysaccharide salts in the form and composition used might well be different from the active principles necessary in the reparative processes. Since gelatin alone does not exhibit a similar effect (unpublished experiments), the active principle presumably is not in the collagen moiety of cartilage. Whether this substance is released from damaged cells or is elaborated by surviving cells in the area of trauma is still to be established. It is possible that the preparations used may contain a substance which inhibits the steroid "pharmacologically." Experiments are continuing in this laboratory with the aim to characterize the hypothetic active material present in these crude suspensions which appears to overcome the blocking effect of cortisone on the reparative phenomena of the connective tissues. Perhaps such studies may be carried out best by tissue culture methods, testing a series of purified mucopolysaccharides and other substances. In support of this is the fact that workers in the field of tissue culture have been aware for many years that fragments of cartilage added to a culture of fibroblasts will stimulate their growth.[5,6] Healy *et al.*[6] demonstrated that at least part of this effect was caused by glucuronic acid.

Summary

Several different crude preparations of cartilage, when injected as suspensions into cortisone-treated animals, were observed to overcome locally the cortisone-induced depression of reparative processes. Similar but much less marked effects were obtained with suspensions of powdered bone, tendon, umbilical cord, and with a preparation containing chondroitin sulfate with gelatin. These findings suggest that cortisone interferes with the reparative processes by blocking the local elaboration or activation of some chemical substance. This chemical factor (or factors) apparently is present in tissues whose ground substance is rich in acid mucopolysaccharides.

We are indebted to Miss Daisy Mapes, R.N., for her efficient and intelligent assistance in all of the animal experimental work.

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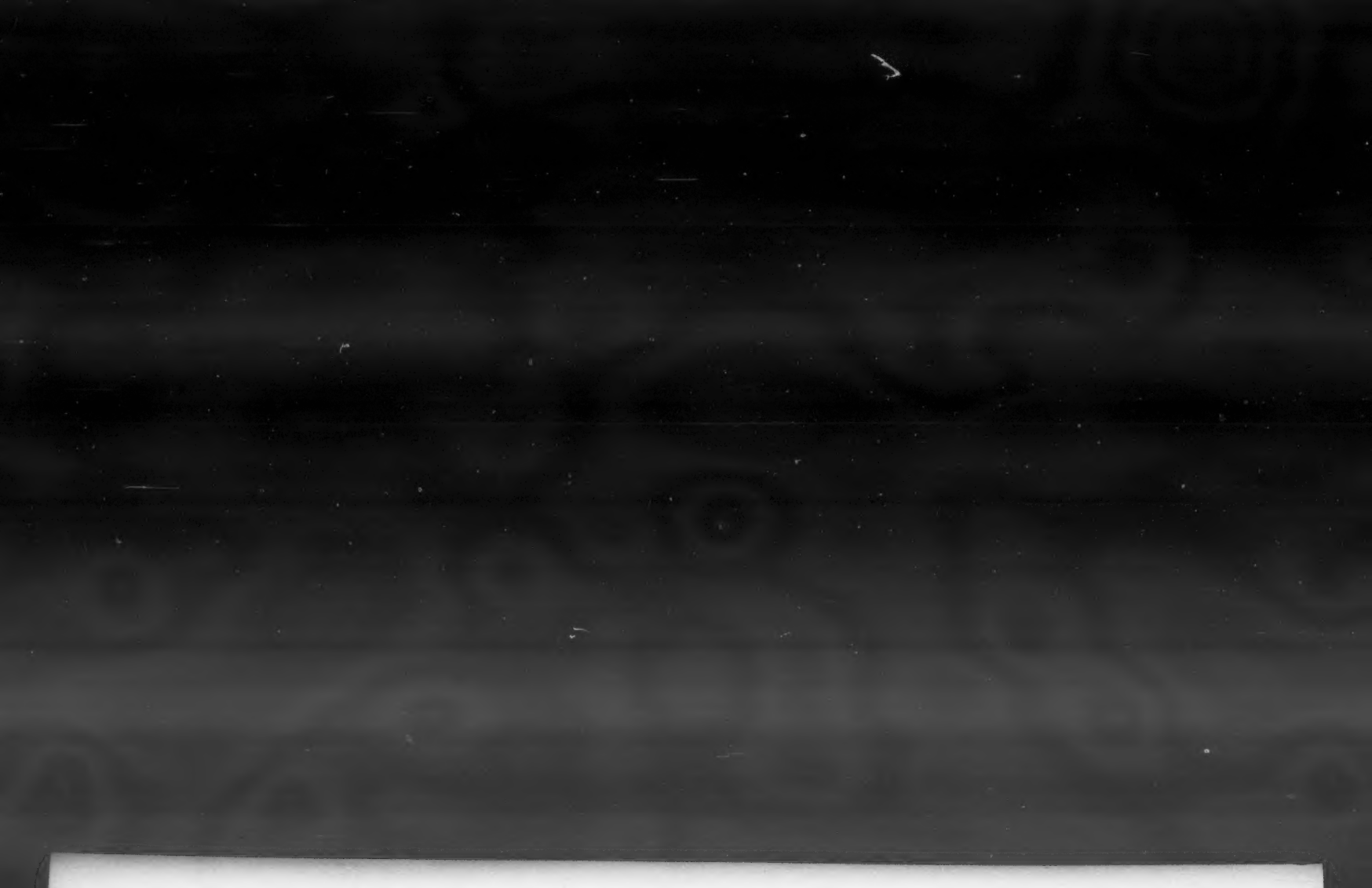
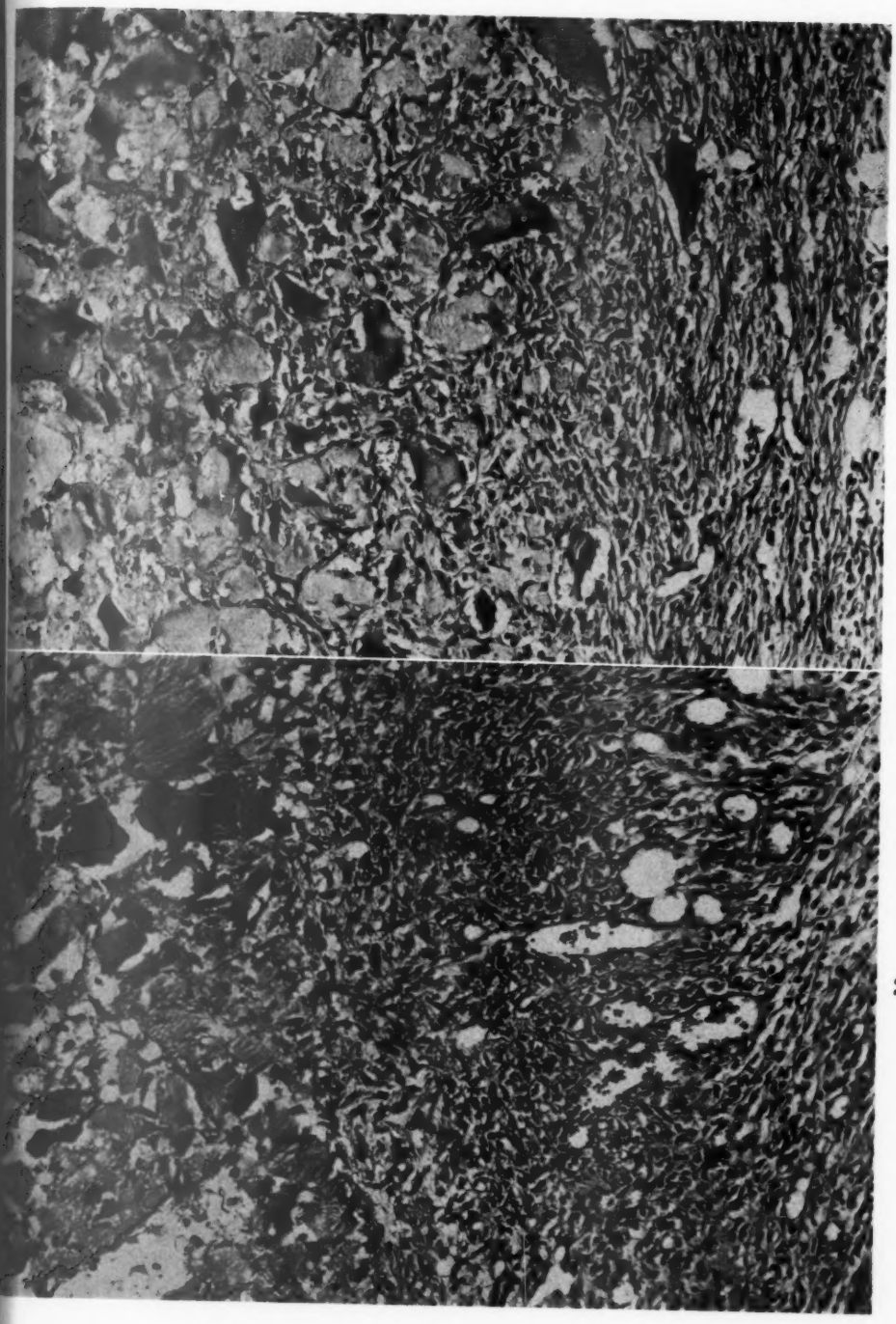
LEGENDS FOR FIGURES

FIG. 1. Zone of granulation tissue associated with implanted pledget of gauze and injections of cartilage suspensions in a cortisone-treated rat, 5 days postoperatively. The cartilage preparation used was autoclaved, pepsin-treated, bovine tracheal cartilage. Hematoxylin and eosin stain. × 165.

FIG. 2. Zone of granulation tissue associated with implanted pledget of gauze and injections of cartilage suspensions in a control rat, 5 days postoperatively. The cartilage preparation used was autoclaved, pepsin-treated, bovine tracheal cartilage. Hematoxylin and eosin stain. × 165.





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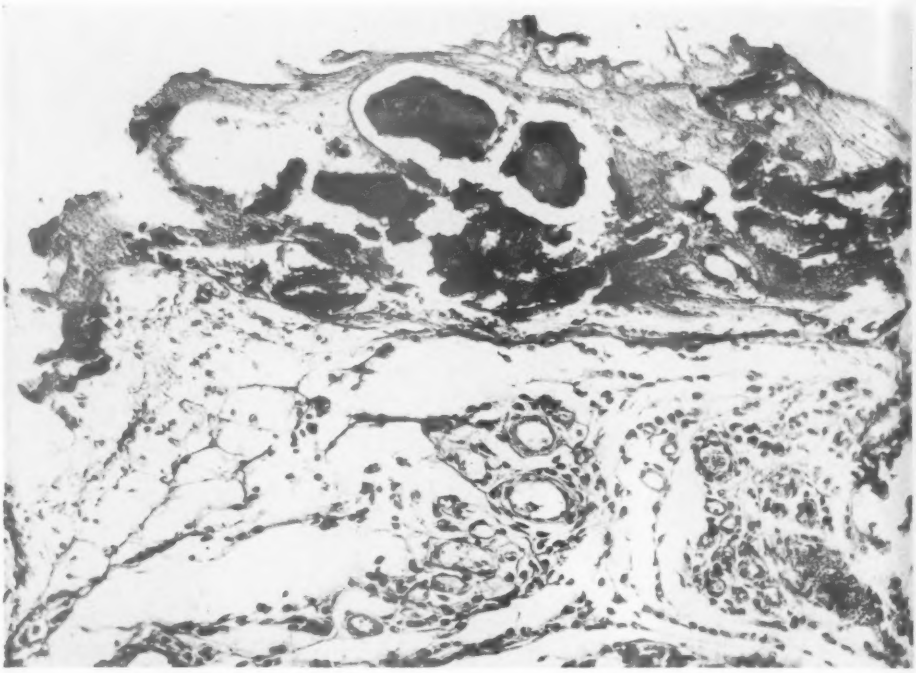
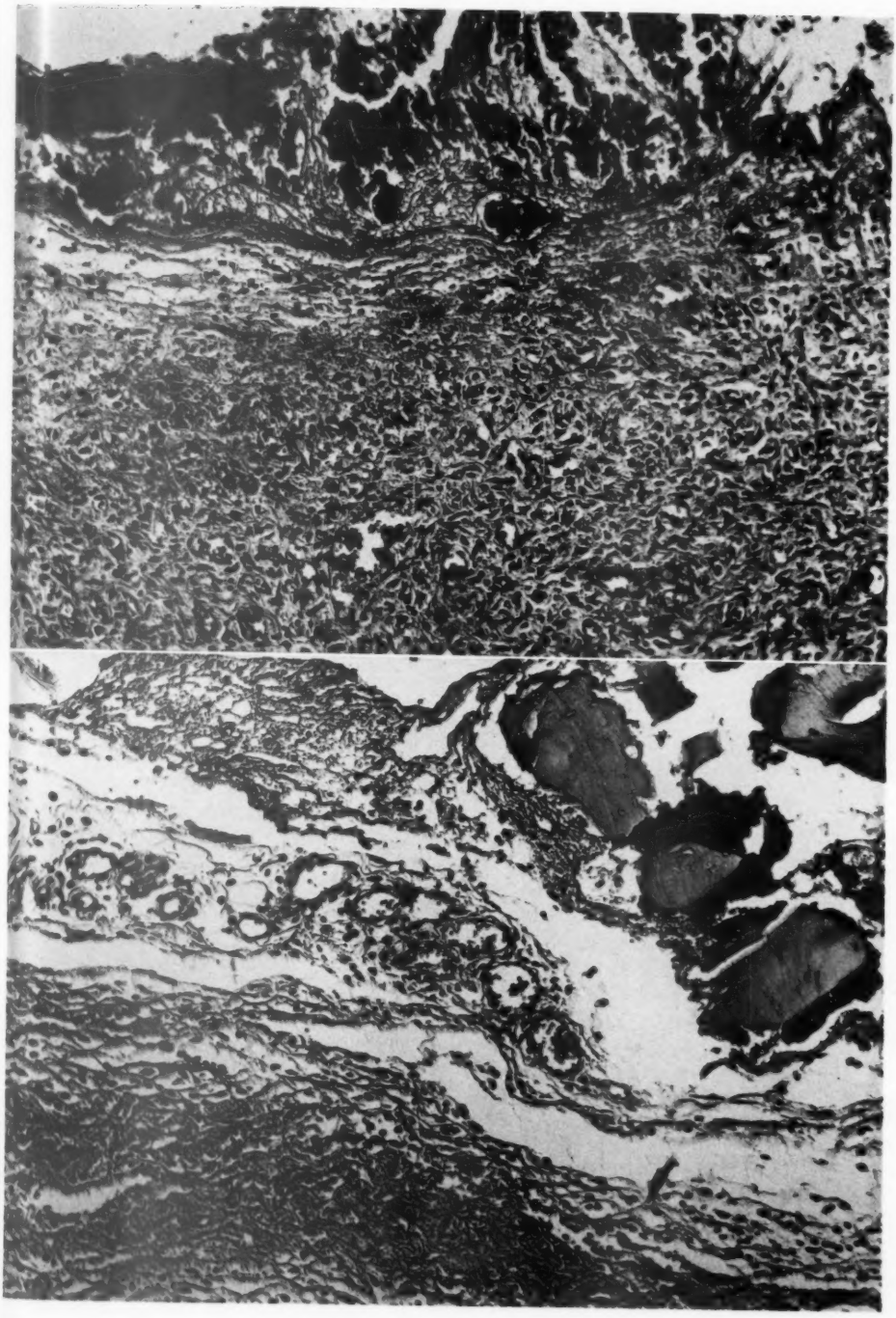


FIG. 3. Two small foci of fibro-angioplasia associated with a pledget of gauze and powdered bone suspension, in a cortisone-treated rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.

FIG. 4. Abundant granulation tissue associated with a pledget of gauze and powdered bone suspension in a control rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.

FIG. 5. Small focus of fibro-angioplasia associated with a pledget of gauze and shredded beef tendon suspension in a cortisone-treated rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.



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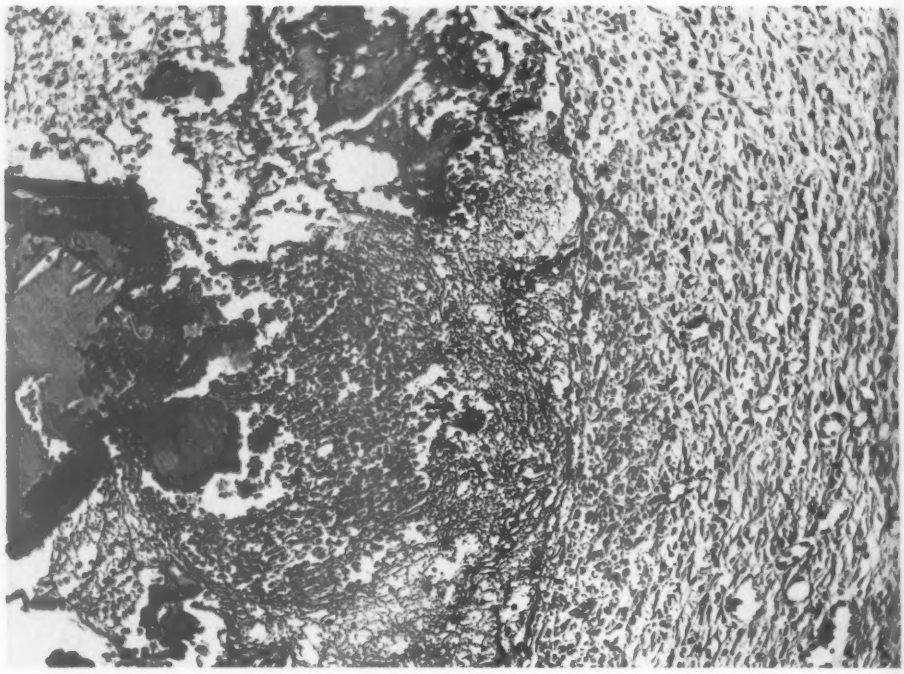

FIG. 6. Abundant granulation tissue associated with a pledget of gauze and shredded beef tendon suspension in a control rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.

FIG. 7. Cortisone-treated rat, 5 days postoperatively. Practically complete lack of reparative response in association with a pledget of gauze in which daily injections of a 2 per cent solution of chondroitin sulfate C were made. Only scattered wandering fibroblasts are seen but no capillary proliferation. The hyalinized fibrinous membrane was a frequent finding. Hematoxylin and eosin stain. × 165.

FIG. 8. Control rat, 5 days postoperatively. Highly cellular, abundant granulation tissue in association with a pledget of gauze into which daily injections of a 2 per cent solution of chondroitin sulfate C were made. The cellularity is due in part to mononuclear and polymorphonuclear leukocytic infiltration. This was seen in most of the animals of this group treated with chondroitin sulfate C. Bacteriologic cultures were negative. Hematoxylin and eosin stain. × 165.

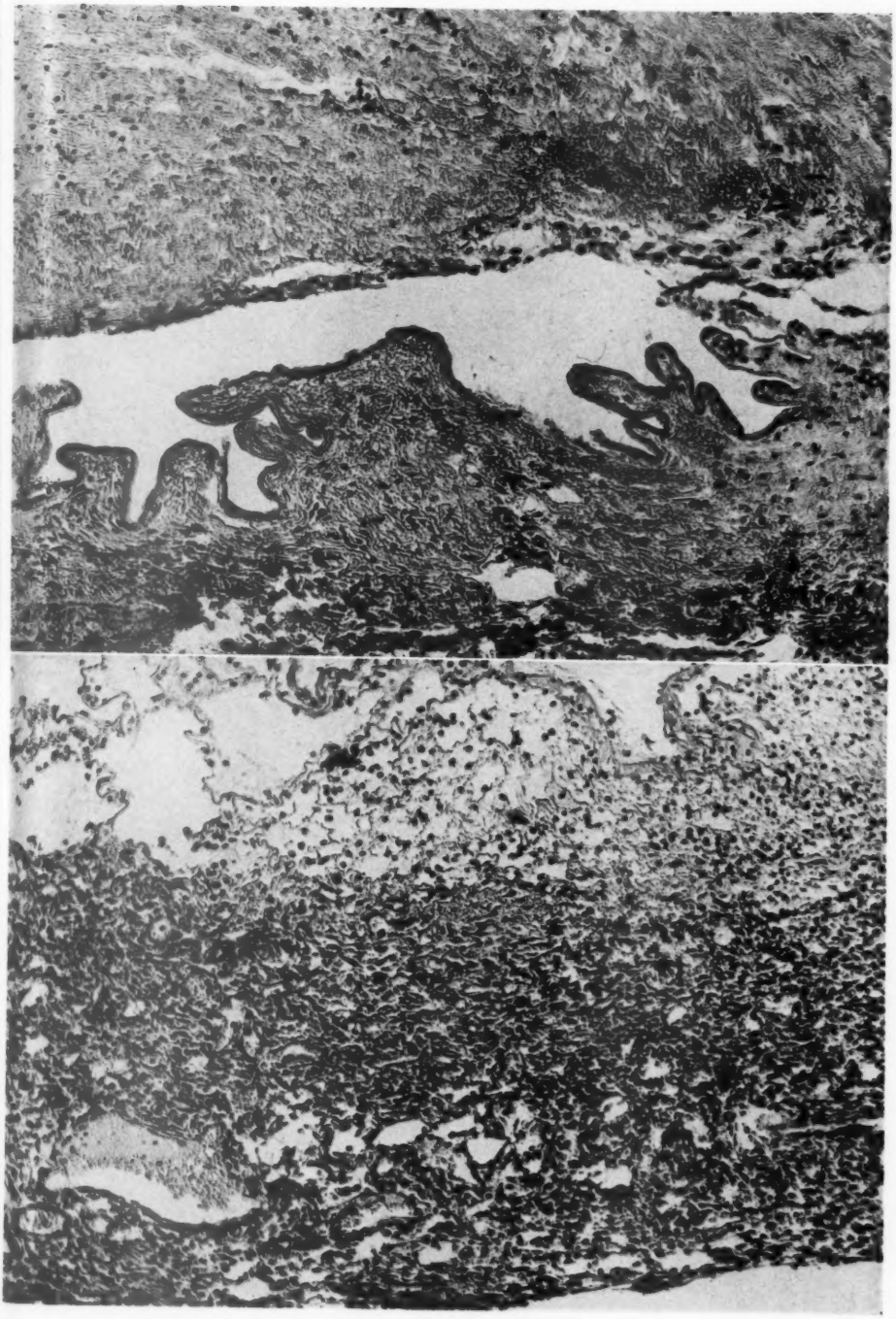

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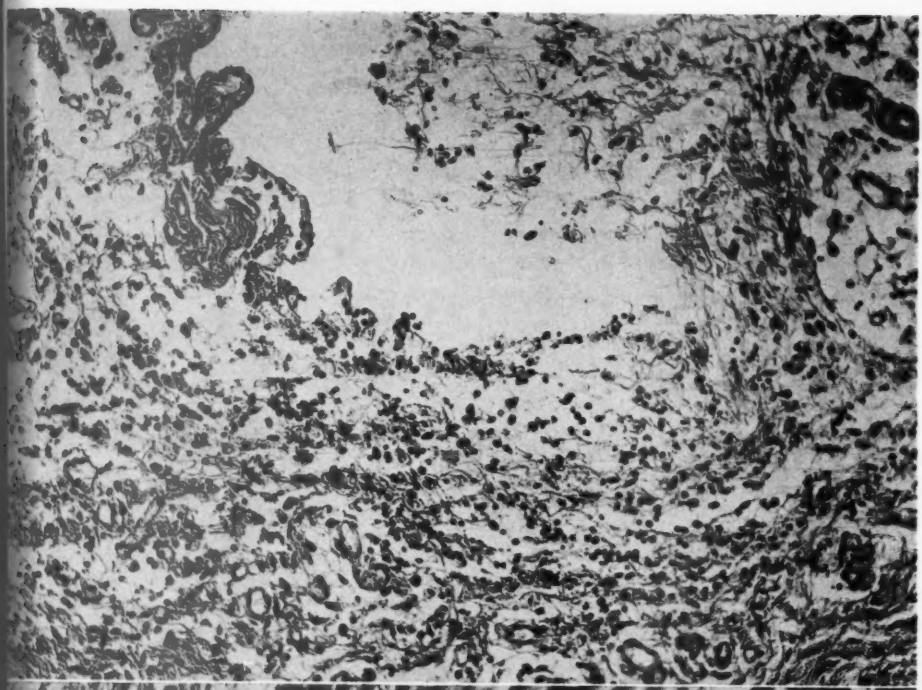
FIG. 9. Cortisone-treated rat, 5 days postoperatively. Tissue surrounding a pledget of gauze into which daily injections of chondroitin sulfate A and gelatin were made. Minimal, sparse, capillary and fibroblastic proliferation is associated with a predominantly mononuclear exudate. Hematoxylin and eosin stain. × 165.

FIG. 10. Cortisone-treated rat, 5 days postoperatively. Tissue surrounding a pledget of gauze associated with a suspension of dried (human) umbilical cord. There is moderate to minimal diffuse fibro-angioplasia. Hematoxylin and eosin stain. × 165.



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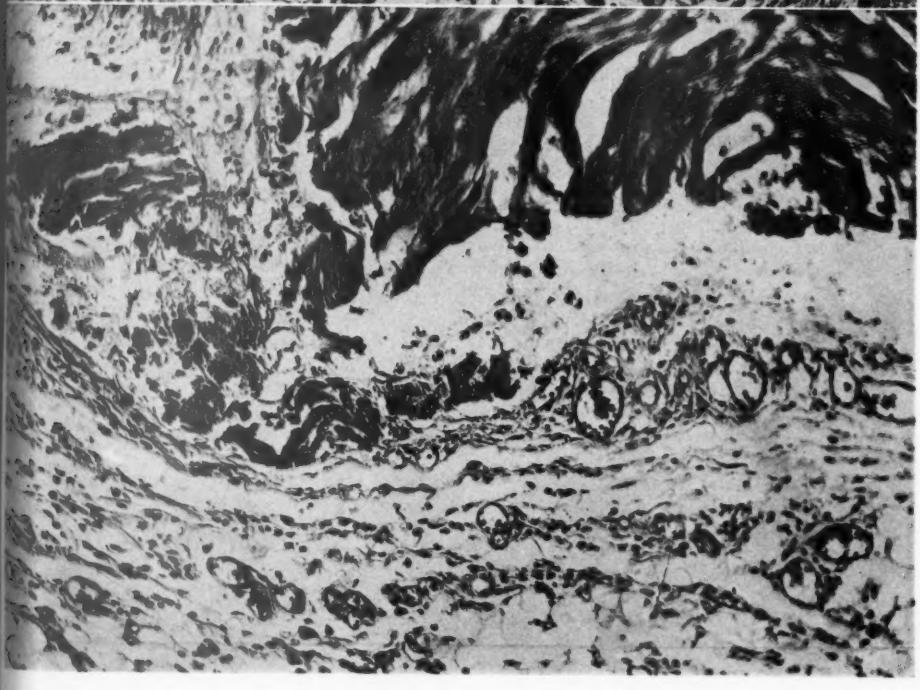

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NECROSIS AND REGENERATION OF SKELETAL MUSCLES IN CORTISONE-TREATED RABBITS*

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Numerous studies have been made of the morphologic changes in experimental animals given ACTH and cortisone,[1-5] and several workers have noted necrosis of skeletal muscle in animals so treated,[6,7] though the lesions have not heretofore been described in detail or illustrated. In the work to be reported, a detailed account will be given of necrosis and regeneration of muscle fibers as observed regularly in rabbits given massive doses of cortisone.

METHODS AND MATERIALS

The muscular lesions developing in cortisone-treated rabbits were studied histologically at various stages and compared with those in the muscles of rabbits on potassium-deficient diets. In addition, chemical analyses for water, lipids, nitrogen, sodium, and potassium were made on the muscles of cortisone-treated rabbits and on the muscles of normal control rabbits. The concentration of potassium in the plasma of the animals of the various groups was determined with a flame photometer. Attempts were made to prevent or alter the development of cortisone-induced muscle necrosis by means of dietary supplements of alpha-tocopherol, or potassium and thiamine. In order to investigate the possible relationship of the muscular lesions to viral or bacterial agents, extracts of skeletal muscle showing extensive necrosis were injected into suckling mice and rabbits and repeated cultures were made of the blood of cortisone-treated rabbits.

Male and female albino rabbits weighing between 0.5 and 1.0 kg. and adult hybrid rabbits ranging from 2 to 3 kg. were used. All were fed Rockland rabbit ration and water, with certain exceptions to be indicated later. The test and control rabbits were examined daily and weighed at frequent intervals. Cortisone acetate was given daily in doses of 10 mg. per kg. into the left anterior thigh muscles of the experimental rabbits. An equal volume of 0.9 per cent NaCl solution was injected into the thigh muscles of the control rabbits.

The rabbits were sacrificed by means of an intravenous injection of sodium pentobarbital or air, and complete post-mortem examinations were made. Muscular tissues from the forelegs, pectoralis major, anterior and posterior thigh groups, psoas major, sacrospinalis, abdominal wall, diaphragm, tongue, heart, urinary bladder, and colon

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were fixed in Zenker-formol solution and sectioned. In some instances, other viscera, together with the peripheral nerves, brain, and spinal cord also were studied microscopically. The tissues were stained routinely with hematoxylin and eosin, and sometimes with eosin and methylene blue, Sudan IV, Marchi's method for fatty degeneration, Masson's stain, and von Kossa's stain for calcium. Sections of peripheral nerves, spinal cord, and brain were stained for myelin.

Development and Character of the Muscular Necrosis in Cortisone-Treated Rabbits

The muscles of 42 rabbits given cortisone for periods of 7 to 25 days were examined, along with those of 60 control rabbits. The skeletal muscles of all animals given cortisone for periods of 14 days or longer were regularly pale, soft, and atrophic; microscopic examination disclosed widespread necrosis and regeneration of muscle fibers, whereas these changes were not present in muscles of the 60 controls. The morphologic alterations will now be described in detail.

Necrosis and Phagocytosis

The earliest recognizable stage in necrosis was swelling of a segment of the muscle fibers, so that these became three to four times their normal size, and stood out in sharp contrast to the adjacent fibers that were often somewhat displaced by pressure (Fig. 1). The swollen segment involved only a portion of the length of the fiber, as could be demonstrated by longitudinal sections in which a relatively long portion of a single fiber was observed. At both ends of the swollen segment, there was either a clearly demarcated and abrupt junction with the normal portion, or in many instances the two portions were separated for various distances, presumably by retraction of the normal muscle from the swollen segment. In such cases, the normal and swollen portions were connected by an empty sarcolemmal tube that was conspicuous because of its narrowed lumen and wrinkled wall. When separation had occurred, the end of the normal muscle fragment was either irregularly frayed or capped by an irregular band of condensed and deeply eosinophilic sarcoplasm. Viewed in cross section, the swollen portion generally had a smooth outline and was contained by an intact but stretched sarcolemmal sheath (Figs. 2 and 3).

The sarcolemmal nuclei usually were unchanged; sometimes they were small and pyknotic. In addition to swelling, a variety of other changes were observed in the structure of the fibers. In some instances, the swollen segment showed single or multiple transverse fractures that resulted in several fragments of various sizes. The

cross and longitudinal striations were distinct in some swollen fibers; in others the protoplasm was homogeneous and glassy, or at times transformed into uniformly small, pale granules (Figs. 1 to 3). The swollen segments stained irregularly with eosin; in phosphotungstic acid preparations stained with hematoxylin, they were uniformly pale pink and stood out in sharp contrast to the normal portions, which were a dark purple-red. The protoplasm of some of the swollen fibers contained many vacuoles of various sizes; these stained red with oil red O. No micro-organisms were found in the swollen fibers in numerous sections stained by the Gram, Giemsa, and Levaditi methods.

The protoplasm of the swollen fibers was obviously necrotic, for it was more or less promptly phagocytized by mononuclear cells, which became conspicuous within and to a much lesser degree around the stretched and sometimes ruptured sarcolemmal tubes (Figs. 3 to 6). The mononuclear cells first appeared in small numbers at the periphery of the necrotic fiber just beneath the sarcolemmal wall and formed a crescent or ring about the necrotic débris (Fig. 3). After removal of the débris, the phagocytes formed solid nests of cells, often containing clearly visible fragments of the eosinophilic sarcoplasm within their cytoplasm (Figs. 3, 4, and 5). An occasional histiocyte was in mitosis as is illustrated in Figure 5.

A thorough investigation of the central and peripheral nervous system was made of 5 animals with extensive necrosis of skeletal muscle following the injection of cortisone, 10 mg. per kg. per day for 3 weeks. Sections taken from the brain, various levels of the spinal cord, and peripheral nerves were stained for myelin and with Masson's stain. No abnormalities were present.

Distribution and Frequency of Muscle Necrosis

The necrosis of segments of individual muscle fibers was quite widespread throughout the body, although the number of altered fibers varied considerably from muscle to muscle and from animal to animal. For example, the frequency with which the fibers were affected in a given muscle, as judged histologically, varied from widespread involvement, as illustrated in Figure 1, to involvement of only a few isolated fibers in an entire microscopic field. A predilection for involvement of a particular skeletal muscle group was not observed, for varying numbers of necrotic fibers were always found in the muscles of the forelegs, anterior and posterior thighs, abdominal wall, psoas, sacrospinalis, chest, diaphragm, and tongue of animals given cortisone for 21 days or longer. It seems significant that the muscular

changes were limited to the voluntary muscles. None of the 42 rabbits given cortisone developed abnormalities in the cardiac muscle or in the involuntary muscle of the urinary bladder, vagina, fallopian tubes, colon, or media of arteries.

In general, the number of necrotic fibers in all muscles increased with the length of cortisone administration; whereas necrotic fibers were not present in the muscles of the forelegs, hind legs, and paravertebral groups of 60 control rabbits. For example, in 5 rabbits that were given cortisone for 5 to 7 days there were only scattered hyaline and swollen muscle fibers in the anterior and posterior thigh muscles, the forelegs, and diaphragm; the remaining muscles appearing essentially normal. The necrotic fibers were more numerous in the muscles of 13 animals given cortisone for 10 to 14 days; furthermore, they were found in the muscles of the forelegs, anterior and posterior chest, anterior and posterior thighs, psoas major, sacrospinalis, diaphragm, tongue, and anterior abdominal wall. Twenty-eight rabbits were given cortisone (10 mg. per kg. per day) for 21 to 25 days; the necrotic fibers were even more numerous than before, at times involving as many as 50 per cent of the fibers in a microscopic field. The degree and extent of phagocytosis closely paralleled the number of necrotic fibers. Much the same was true of regeneration, as will be shown in the next section.

Regeneration of Muscle Fibers

Regeneration generally began before phagocytosis was completed and was conspicuous in all 35 rabbits given cortisone for 14 days or longer. The regenerative process consisted of an outgrowth within the sarcolemmal tube of a conical or irregular mass of basophilic cytoplasm continuous with, and apparently arising from, the uninjured portions of the intact fiber and sarcolemma on either side of the necrotic segment (Figs. 7 and 8). The regenerating, basophilic, and granular tip was devoid of cross or longitudinal striations and showed conspicuous proliferation of muscle nuclei which were often aligned in parallel rows (Fig. 8). When phagocytosis of the necrotic segment was incomplete, the regenerating tip often formed a blunt or scalloped, multinucleated mass, abutting the necrotic segment or extending partially or completely around the necrotic segment as an extremely fine band (Fig. 8). In addition to these changes, there was proliferation of the cells that formed the walls of the sarcolemmal tubes. These cells were markedly flattened, had scanty basophilic cytoplasm, and large basophilic nuclei containing one or two nucleoli (Fig 5). As is

shown in Figure 9, many other fibers, apparently in a later stage of regeneration, were conspicuous because of a variable but generally small diameter, a tubular shape, centrally placed nuclei, and basophilic cytoplasm. In some of these the sarcoplasm in the central portion of the fiber was basophilic, granular, and completely lacking in cross striations (Fig. 9).

In addition to the histologic observations which indicated that active regeneration was taking place in the muscles of the cortisone-treated rabbits, the following observations show that complete healing of the muscle lesions occurred after the cortisone injections were discontinued. Each of 5 rabbits given cortisone for 21 days manifested segmental necrosis of voluntary muscle fibers, as was determined by biopsies of the sacrospinalis muscle made at the end of this period. When the animals were sacrificed 6, 12, 16, 21, and 22 weeks after the cortisone had been discontinued, their muscles in every instance were histologically normal. Fibrosis was absent.

The findings make it clear that rabbits given large quantities of cortisone intramuscularly develop widespread segmental necrosis of the skeletal muscle fibers, phagocytosis of the débris, and regeneration of the involved fibers. A few necrotic fibers were noted 5 days after cortisone injections were started; regenerating and necrotic fibers were conspicuous after 14 days or longer. Regeneration of the fibers apparently was complete, for the involved muscles appeared normal after an interval if cortisone injections were stopped. The muscular lesions were limited to the voluntary or skeletal muscle fibers; the significance of the absence of cardiac necrosis will be discussed in reference to experimental potassium deficiency in the rabbit.

Other Changes

Hyperlipemia, fatty infiltration and focal necrosis of the liver, together with atrophy of the adrenal cortex and lymphoid tissue, and inhibition of growth were conspicuous in the cortisone-treated rabbits, as has been noted previously by others.[1-3,5,7] Growth of the rabbits was greatly diminished during the period of cortisone administration, and those given cortisone for 7 days or longer all lost weight. The cortisone-treated animals (10 mg. per kg. per day) gained an average of 16 gm. per day for the first 7 days, after which time they lost an average of 15 gm. per day. Control rabbits gained an average of 21 gm. per day. Food, water, and urinary volume of 5 cortisone-treated rabbits were measured daily and the observations showed that weight loss occurred while the food intake was equal to or greater than it was before cortisone injections were given. During this period water consumption and urine volume were increased to one to two times those of the control rabbits. A controlled observation was made to test the effect of weight loss on the structure of muscular tissue. Six normal, adult, hybrid animals were placed on a reduced dietary intake so that 30 to 35 per cent of the body weight was lost in 14 to 21 days. Histologic sections showed atrophy of muscle fibers but absence

of segmental necrosis of the muscle fibers such as were found in the cortisone-treated animals. Furthermore, it was observed that these animals did not manifest as marked muscular weakness or tremor as did the cortisone-treated animals.

After 4 to 6 days of cortisone administration, there was marked distention of the abdomen, a condition which continued throughout the experiment. Subsequent post-mortem examination revealed this to be due to gaseous distention of the intestines and enlargement of the liver. After injections were given for 1 week, there was a roughening of the normally soft fur of the animals. Stiffness of the joints was not present.

Chemical Alterations in the Muscles of Cortisone-Treated Animals

Analyses for water, sodium, potassium, nitrogen, and total lipids were made of muscles with cortisone-induced necrosis because cortisone is known to affect the metabolism of these substances.

The muscles from the anterior and posterior thighs, forelegs, and sacrospinalis groups of each of 6 immature rabbits given cortisone (10 mg. per kg. per day) for 10 to 25 days were minced and pooled, samples of each then being dried at 110°C. until a constant weight was reached. The muscular tissues of each of 6 normal control rabbits were treated similarly. Lipids were extracted with ethyl and petrol ether, and then sodium and potassium were liberated from the dry fat-free residue by the method of Lowry and Hastings.[8] Sodium and potassium analyses were performed by means of a flame photometer. Analyses for total nitrogen were made on the dried fat-free residue by the macro-Kjeldahl technique. Values were expressed in terms of 100 gm. of dried fat-free muscle residue. The heart muscle was analyzed in a similar fashion, except that nitrogen determinations were omitted.

As is shown in Table I, the muscles with the cortisone-induced necrosis, in contrast to the normal controls, contained moderately increased amounts of water (an average of 351 gm. of water could be extracted from normal tissues yielding 100 gm. of dried fat-free solids, whereas the altered muscles yielded an average of 423 gm. of water), and markedly increased amounts of lipid (an average of 3.5 gm. was extracted from the normal muscle yielding 100 gm. of dried fat-free solid, while an average of 19.6 gm. was extracted from the muscle with necrosis). Furthermore, the potassium content of the muscle with necrosis was diminished (an average of 42.6 mEq. per 100 gm. of dried fat-free solids in the altered muscle as contrasted to 53.5 mEq. in the normal controls) and the sodium content correspondingly increased (27.1 mEq. in the muscle with necrosis and 9.1 in the normal muscle), so that the sum of these cations equaled approximately that of the normal controls. The values for total nitrogen content of the samples of normal muscle and of muscle with the cortisone-induced necrosis were equal, the figures indicating that the diminution in potassium was not merely secondary to a reduction in protein content of the sample.

The cardiac muscle from the same animals failed to reveal signifi-

cant abnormalities in water, lipid, sodium or potassium content, this fact having greater significance since necrosis was never manifest in the hearts of the cortisone-treated animals.

Abnormalities in muscle electrolytes, lipid, and water content have been found in several of the conditions, which show morphologic

TABLE I

Studies on Skeletal Muscle of Cortisone-Treated Rabbits

Experimental			Chemical composition of skeletal muscles					
Groups	Rabbit	Treatment	Water	Lipid	Nitrogen	Sodium	Potassium	Muscle necrosis
	no.	*days*	*gm.**	*gm.**	*gm.†*	*mEq.†*	*mEq.†*	
I. Control rabbits	1664		344	5.4	14.7	8.5	52.6	Nil
	1665		346	2.3	14.6	8.1	54.5	Nil
	1666		349	3.2	14.6	8.5	52.3	Nil
	1678		356	5.6	14.5	10.9	54.8	Nil
	1679		356	1.8	14.4	9.8	51.6	Nil
	1682		358	2.8	14.3	8.7	54.9	Nil
		Averages:	351	3.5	14.5	9.1	53.5	
II. Cortisone-treated rabbits‡	1680	10	400	8.1	14.5	27.7	43.2	Slight
	1677	17	367	13.5	15.0	26.1	38.0	Severe
	1681	22	437	11.5	14.2	22.3	38.4	Severe
	1773	25	470	32.6	14.2	33.0	47.4	Moderate
	1774	25	418	23.2	14.2	27.0	46.4	Moderate
	1775	25	445	28.8	13.8	26.8	42.8	Moderate
		Averages:	423	19.6	14.3	27.1	42.6	

* Amount extracted from tissue yielding 100 gm. of dried fat-free solids.
† Amount contained in 100 gm. of dried fat-free solids.
‡ Cortisone acetate given in doses of 10 mg./kg./day.

changes similar to those found in cortisone-treated rabbits. For example, an increase in sodium content and decrease in potassium have been reported in the muscles of vitamin E-deficient rabbits,[9] in scorbutic guinea-pigs,[10] in potassium-deficient animals,[11] and in human polyneuritis.[12] Furthermore, diminished potassium values (sodium analyses not performed) have been reported in the muscles of mice infected with Coxsackie virus,[13] and in atrophic muscles following nerve section.[14] Therefore, the decreased potassium and increased sodium content of muscle of cortisone-treated rabbits is not specific and it seems likely that the electrolyte changes are at least partially secondary to a reduction in the normal muscle cell mass due to atrophy and necrosis of the muscle fibers and to expansion of the extracellular space. Whether or not actual displacement of potassium by sodium

occurred in the muscle fibers is not shown by the histologic or chemical findings. Increases in lipids have been observed in the muscles in vitamin E deficiency,[15] in polyneuritis,[12] as well as in the cortisone-induced necrosis. The exact significance of increased lipid content is poorly understood, but it seems unlikely that such changes are primary since accumulations of lipids are common in damaged tissues.

Studies of the Relation of Cortisone-Induced Muscular Necrosis to Potassium Deficiency, Vitamin E Deficiency, and Infection

Potassium Deficiency

A comparison of the cortisone-induced muscular necrosis with experimental potassium deficiency was made, since the potassium content of the muscles in cortisone-treated rabbits was diminished, and potassium deficiency in other species has been reported to cause necrosis of skeletal muscle, though not heretofore in rabbits.[16,17] Furthermore, previous studies by Milman and Milhorat[18] of the metabolic patterns of immature rabbits given the same amount of cortisone showed a somewhat increased urinary excretion of potassium.

Nine male and female hybrid rabbits, weighing from 2 to 3 kg., were placed on a low potassium diet supplied by the National Biochemical Corporation and containing 64.2 per cent cornstarch, 30.0 per cent casein, 3.5 per cent butter fat, 1.3 per cent calcium carbonate, 1.0 per cent sodium chloride, and vitamin supplements. The cardiac and skeletal muscles were studied after varying intervals.

All rabbits on the deficient diet developed severe muscle weakness and 8 of them died between the 7th and 19th days. The remaining animal was sacrificed after being on the deficient diet for 8 weeks. The voluntary muscles of 3 rabbits revealed a fine, linear, yellow-white streaking and focal gray areas of necrosis in the myocardium of the left ventricle and interventricular septum.

Myocardial necrosis, as shown microscopically, was found in every animal and varied from necrosis of a few isolated subendocardial fibers to necrosis of up to 50 per cent of the ventricular wall with extensive calcification and mural thrombosis. In all animals, there was segmental necrosis, phagocytosis, and regeneration of voluntary muscle fibers, indistinguishable from that in the cortisone-treated rabbits. This varied from isolated necrotic fibers in muscle groups of the fore and hind legs, the trunk, the paravertebral groups, and tongue in 3 rabbits to necrosis of many fibers in 6 rabbits.

To learn more about the relationship of potassium to the cortisone-induced necrosis, the diets of cortisone-treated rabbits were supplemented with potassium and thiamine since deficiencies of these substances are known to produce skeletal muscle necrosis[19,20] and oral supplementation of potassium has been shown to prevent the electrolyte changes, muscle lesions, and paralysis induced by potassium deficiency.[16,21,22]

Five immature albino rabbits, weighing between 700 and 809 gm., were given cortisone acetate (10 mg. per kg. per day) into the left anterior thigh muscles for

21 days. The animal also received 2 mg. of thiamine hydrochloride intramuscularly each day. Their drinking water contained 1 per cent potassium chloride and was offered at all times. Five similar control rabbits were given cortisone in the same dosage and received tap water to drink. The animals in each group consumed approximately 300 cc. of fluid daily. Both groups were offered Rockland Rabbit Ration pellets at all times. These contain 1.5 per cent potassium.

Each of the 5 rabbits receiving potassium and thiamine supplements developed lesions of voluntary muscle indistinguishable from those in rabbits whose diet was not so supplemented. The general appearance of the animals and other findings in both groups were identical and the same as those described in detail in a previous section.

To explore further the relationship of potassium to the cortisone-induced muscle necrosis, the concentration of plasma potassium was determined in rabbits injected with cortisone, that had been given either tap water or 1 per cent potassium chloride as a sole source of fluid, as well as in control groups of normal rabbits that were given similar fluids but were not injected with cortisone.

Five hybrid rabbits of both sexes, weighing from 600 to 1000 gm., were given cortisone acetate (10 mg. per kg. per day) intramuscularly as a single injection and were offered tap water ad libitum. A second similar group received the same amount of cortisone but were given 1 per cent potassium chloride as the sole source of fluid. Control groups consisted of 5 normal animals of the same weight that received 1 per cent potassium chloride as a sole source of fluid, and 15 normal rabbits given tap water. The various experimental procedures were continued for 3 weeks and then the animals were bled from the heart, and plasma potassium concentrations were determined by a flame photometer, using lithium chloride as an internal standard. The animals were sacrificed and complete post-mortem examinations were performed.

The plasma potassium concentrations were as follows: 15 normal rabbits—average, 5.0 mEq. per liter (range, 4.1 to 5.9); 5 normal rabbits given 1 per cent potassium chloride as a sole source of fluid—average, 5.3 mEq. per liter (4.8, 5.0, 5.2, 5.8, and 5.9, respectively); 5 rabbits given cortisone intramuscularly and tap water to drink—average, 4.6 mEq. per liter (3.7, 3.8, 4.6, 5.4, and 5.4, respectively); 5 rabbits given cortisone intramuscularly and 1 per cent potassium chloride as a sole source of fluid—average, 5.3 mEq. per liter (4.9, 4.9, 5.0, 5.7, and 5.8, respectively). The observations indicated that the plasma potassium values in the animals given cortisone with or without oral supplementation with potassium were not significantly different from the control values.

Histologic studies showed that each of the animals receiving cortisone developed widespread necrosis of muscle fibers. The lesions were indistinguishable in the groups receiving water and 1 per cent potassium chloride as sole sources of fluid. The control animals failed to show muscle necrosis.

These experiments show that rabbits fed a potassium-deficient diet develop widespread necrosis of skeletal muscle fibers indistinguishable from that found in the muscles of rabbits given repeated injections of cortisone. In contrast, the rabbits on a potassium deficient diet uniformly showed myocardial necrosis, whereas the cortisone-treated rabbits did not. Supplementation of the diet of the cortisone-treated rabbits with potassium in large amounts failed to prevent or alter the segmental necrosis of skeletal muscle fibers. Furthermore, the concentration of plasma potassium of animals given cortisone with or without supplementation of potassium chloride did not differ significantly from that of normal controls.

Vitamin E Deficiency

The diet of 5 cortisone-treated rabbits was supplemented with large amounts of alpha-tocopherol, since a deficiency of this substance is known to produce muscular necrosis.[23]

Five immature albino rabbits, weighing 700 to 1,000 gm., were given 100 mg. of alpha-tocopherol both orally and subcutaneously for 1 week preceding cortisone administration in order to saturate the body with alpha-tocopherol. Thereafter, during cortisone administration (10 mg. per kg. per day), 12.5 mg. of alpha-tocopherol was given both orally and subcutaneously three times weekly. One animal died on the 10th, 12th, and 17th days, respectively. The remaining 2 were sacrificed after 21 days of cortisone administration.

All 5 rabbits receiving alpha-tocopherol supplements showed muscle lesions that were identical morphologically with those found in the muscles of rabbits not given such supplements and that received cortisone for similar lengths of time.

Infection

Because of the well known effect of cortisone administration on infection and resistance,[24-26] repeated cultures were made of the blood of cortisone-treated rabbits and attempts were made to isolate a viral agent from their necrotic muscles.

Five male and female hybrid rabbits weighing from 600 to 1,000 gm. were given injections of cortisone acetate (10 mg. per kg. per day) for 21 days. During this period samples of blood from the ear of each animal were taken on alternate days, and the heart's blood was obtained on the 7th, 14th, and 21st days and cultured on Todd-Hewitt broth made with neo-peptone. After 2 and 7 days of incubation, the broth cultures were plated on peptone agar enriched with fresh rabbit blood. The cultures failed to show growth of bacteria.

The animals were sacrificed on the 21st day and representative samples of the muscles of the forelegs, hindlegs, psoas, and paravertebral groups were procured under aseptic conditions. (Subsequent study of the microscopic sections of the muscles of each animal showed extensive necrosis of the muscle fibers.) A homogenate of the muscle was prepared in a Waring blendor using 37 gm. of pooled muscle and 250 cc. of 0.9 per cent saline solution. The homogenate was placed in a refrig-

erator and allowed to settle for 2 hours, and the supernatant fluid used for injection. One cc. of the suspension was injected subcutaneously and 0.5 cc. injected intraperitoneally into each of 10 hybrid rabbits that were 11 days old. Of the muscle homogenate, 0.05 cc. was injected both intraperitoneally and subcutaneously into each of 20 C_3H mice that were 3 days of age. The suckling rabbits and mice failed to develop symptoms, and one half of each group were sacrificed 1 week after injection of the muscle homogenate, and the remainder, 2 weeks after injection. Complete post-mortem examinations were performed. Microscopic sections of the viscera of each animal failed to show lesions.

Using Todd-Hewitt media for bacteria and Schuffner's media for spirochetes, attempts were made to isolate organisms from the homogenate of the muscle procured from the rabbits with cortisone-induced necrosis. These cultures were negative. Dark-field examination of the homogenate failed to disclose organisms.

Discussion

Widespread necrosis and regeneration of segments of individual skeletal muscle fibers regularly followed repeated injections of cortisone in rabbits in the experiments here reported. Histologic studies indicated that the skeletal muscles returned to normal after an interval if the cortisone injections were stopped. The mechanism by which cortisone induces these striking changes in skeletal muscle remains unknown.

Experiments were reported, however, which showed that the muscular lesions in the rabbits given cortisone were not secondary to a conditioned deficiency of potassium; for the plasma potassium concentration of the cortisone-treated rabbits was not notably reduced, nor was the muscular necrosis altered by the administration of large amounts of potassium, though oral potassium prevents the development of muscular changes in animals with potassium deficiency induced in other ways.[16,21,22] Furthermore, the cortisone-treated rabbits never developed cardiac necrosis, whereas this was a prominent and regular finding in other groups of rabbits with potassium deficiency induced by dietary means.

The possibility that the cortisone-induced muscular necrosis was secondary to activation of a latent viral infection should be considered, since, experimentally, certain virus infections, such as the Coxsackie and poliomyelitis viruses, that show morphologically similar muscle lesions, may be enhanced by cortisone.[25,26] The reported experiments do not exclude this possibility but make it seem unlikely since homogenates of the muscle procured from rabbits with cortisone-induced necrosis were not pathogenic for suckling mice and rabbits. Repeated cultures of the blood of cortisone-treated rabbits failed to disclose bacteria.

In 1863, Zenker[27] described alterations of voluntary muscles in

patients dying of typhoid fever which were morphologically similar to those found in the muscles of rabbits given large amounts of cortisone. Since that time, similar changes have been reported in humans dying with a variety of acute infectious diseases,[28] myasthenia gravis,[29] muscular dystrophy,[30] dermatomyositis,[31] lupus erythematosus, and other diseases.[32] Experimentally, such lesions occur with deficiencies of vitamin E, thiamine, ascorbic acid, vitamin A, potassium, and antistiffness factor[16,19,23,33-35]; in C3H mice fed purified low protein diets[36]; in rats fed a diet of dried egg white[37]; in herbivora fed various synthetic diets[38,39]; in rats given an excess of vitamin D[40]; in animals given papain intravenously[41]; in rats given plasmocid intraperitoneally[42]; in various viral infections[26,43,44]; and in animals traumatized by a wide variety of physical and chemical agents.[45-49] In spite of the increased knowledge of the distribution of muscular lesions in various human and experimental diseases, little is known of the etiology and pathogenesis of muscular necrosis. Likewise, virtually nothing is known of the factors which determine whether voluntary, cardiac, or involuntary muscles, individually or in combination, will undergo necrosis in any given set of circumstances in which muscular necrosis occurs. The occurrence of a morphologically similar type of necrosis of segments of individual muscle fibers in a wide range of animal species, which is induced by such a large number of apparently unrelated agents, suggests that the lesion is not specific but rather a basic reaction of muscle to injury, and analogous perhaps to coagulation necrosis of other tissue cells.

Summary

Rabbits given large amounts of cortisone acetate for 7 to 25 days regularly developed widespread necrosis and regeneration of skeletal muscle fibers. Complete restitution of normal structure occurred after cortisone was discontinued. In contrast to the skeletal muscle, the cardiac muscle and involuntary muscles of the cortisone-treated rabbits appeared normal.

Chemical analyses of muscle showing cortisone-induced necrosis revealed an increased amount of water, lipid, and sodium, and a diminution in the amount of the potassium, as compared with normal muscle.

Morphologically, the cortisone-induced necrosis of skeletal muscle resembled that seen in rabbits on potassium-deficient diets, though the cortisone-treated rabbits never exhibited myocardial necrosis while the rabbits on potassium-deficient diets did so regularly. Further-

more, the plasma potassium concentration was not notably decreased in the cortisone-treated rabbits and supplementation of the diet of the animals with large amounts of potassium failed to alter the development of muscular necrosis. Likewise, alpha-tocopherol did not prevent the cortisone-induced muscular lesions.

Homogenates of muscle showing the cortisone-induced necrosis were injected into suckling mice and rabbits. These animals remained healthy; when sacrificed their tissues appeared normal. Repeated cultures of the blood of rabbits given cortisone failed to show growth of bacteria.

The actual mechanism by which this striking morphologic lesion is produced remains unknown.

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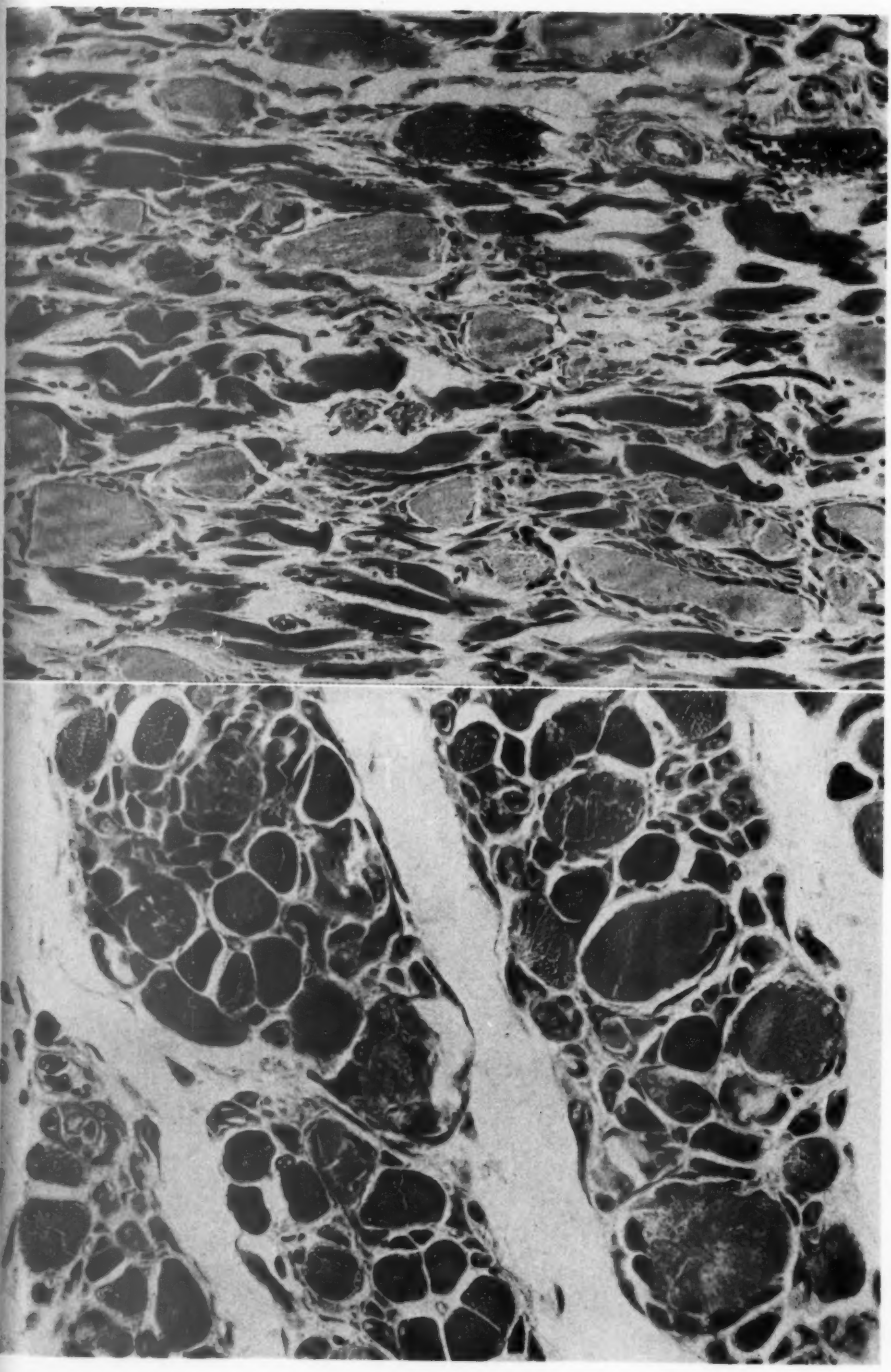
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Muscle of right anterior thigh of rabbit given cortisone (10 mg. per kg. per day) for 14 days, illustrating swelling of segments of the muscle fibers to three to four times the size of adjacent fibers. The swollen fibers are pale and there is almost complete absence of interstitial inflammation. Eosin and methylene blue stain. × 220.

FIG. 2. Cross section of muscle from right posterior thigh of rabbit given cortisone for 21 days, demonstrating several stages in the necrotizing process, including swelling of fibers, accumulation of histiocytes within the sarcolemmal sheaths and to a lesser degree in the interstices, and atrophy and separation of muscle fibers, presumably by edema fluid. Eosin and methylene blue stain. × 540.



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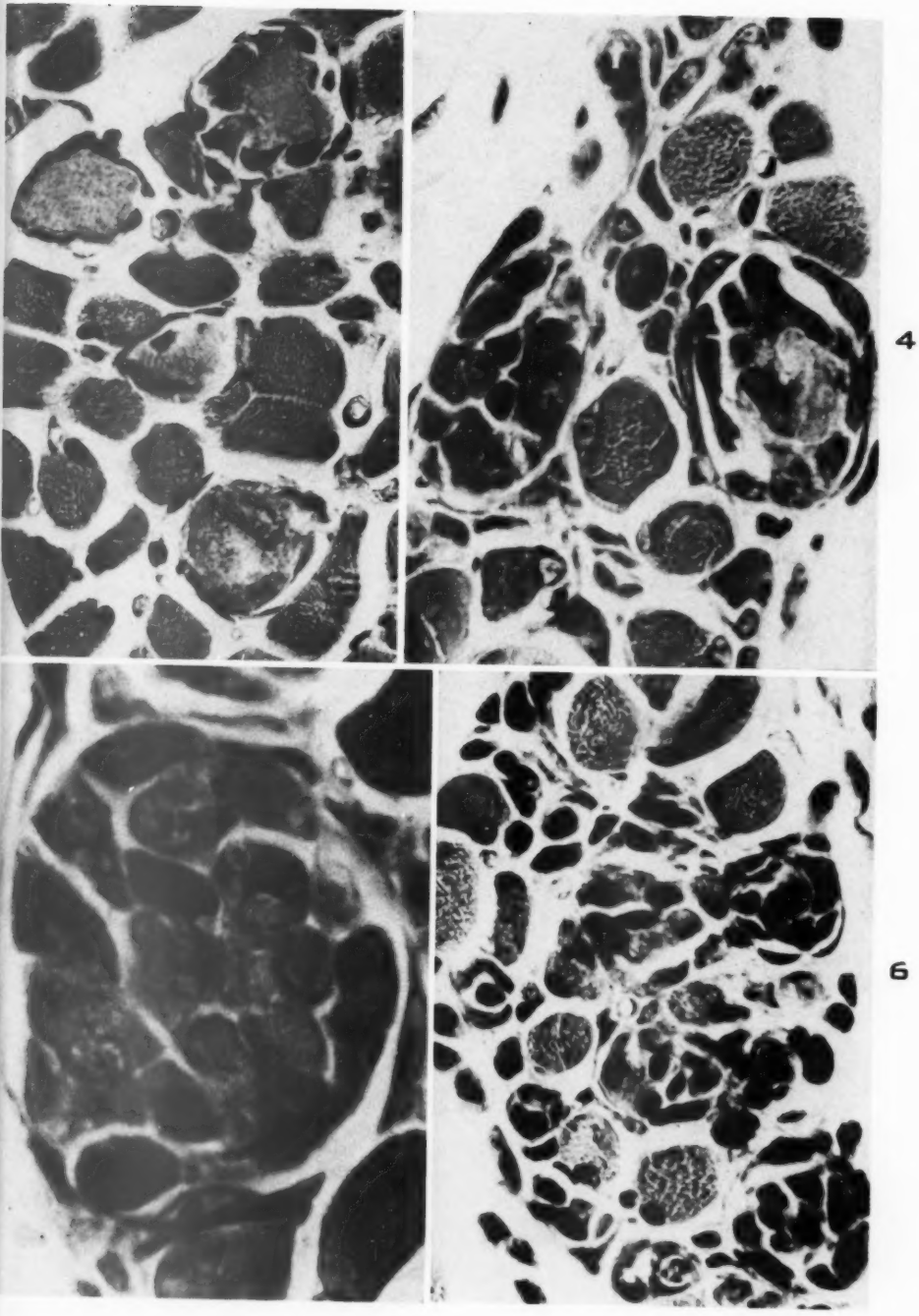
Figures 3, 4, 5, and 6 are taken from the muscles of a rabbit given cortisone (10 mg. per kg. per day) for 21 days and show various stages in the phagocytosis of the necrotic, swollen fibers.

FIG. 3. Early accumulation of phagocytes within the sarcolemmal sheaths of one of four swollen fibers. Longitudinal striations are present in the upper three of these. Hematoxylin and eosin stain. × 600.

FIG. 4. Greater detail of two dilated sarcolemmal tubes; one contains only histiocytes and the other contains histiocytes surrounding necrotic débris. There is moderate separation of the atrophic muscle fibers by interstitial edema fluid. Eosin and methylene blue stain. × 970.

FIG. 5. Dilated sarcolemmal tube containing histiocytes, several with cytoplasmic vacuoles of eosinophilic débris. Large, flattened, sarcolemmal nuclei contain prominent nucleoli. The cell in mitosis in the lower portion of the tube was considered to be a histiocyte. Eosin and methylene blue stain. × 1500.

FIG. 6. Accumulation of histiocytes within the interstitial space and within sarcolemmal tubes. Hematoxylin and eosin stain. × 600.



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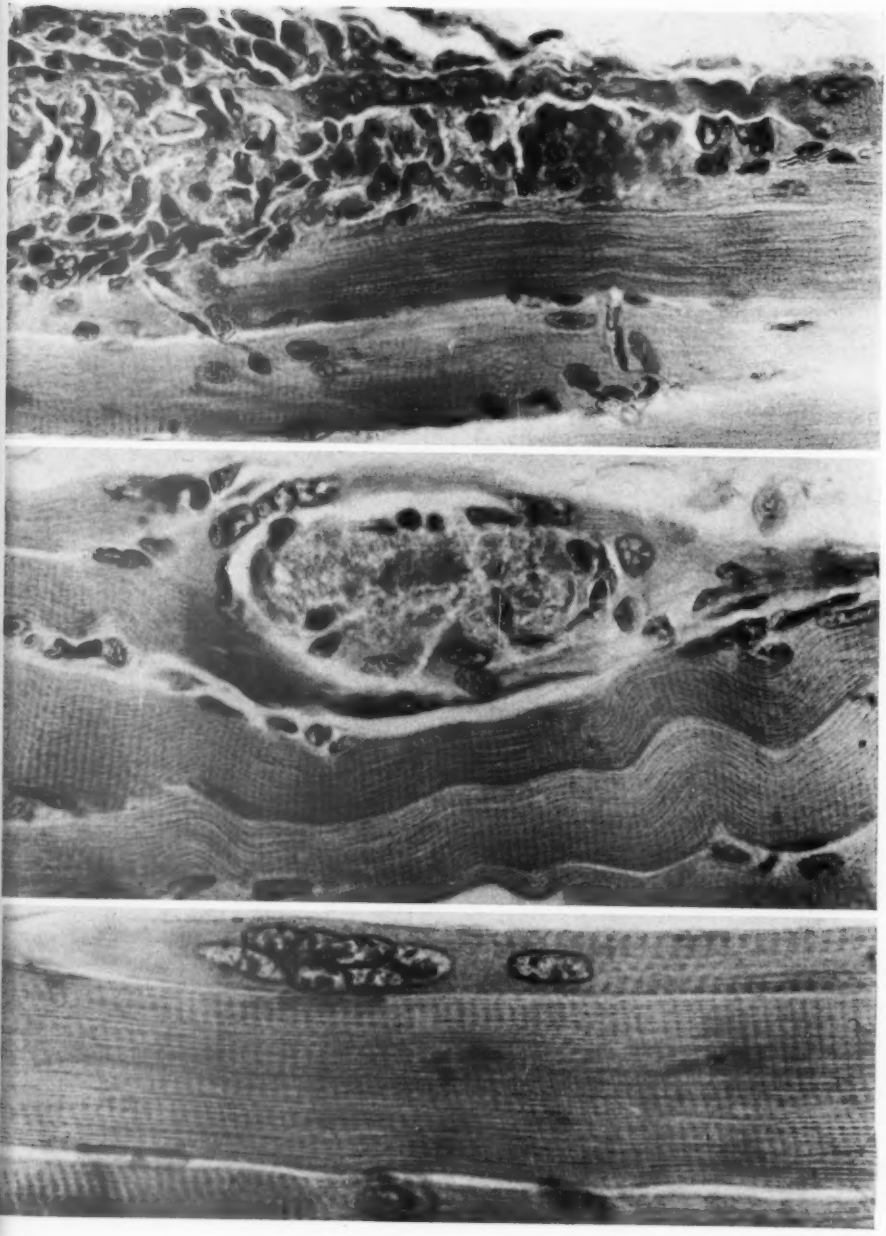
Figures 7, 8, and 9 were taken from the muscles of another rabbit given cortisone (10 mg. per kg. per day) for 21 days, and show various stages in the regeneration of muscle fibers.

FIG. 7. Muscle from right foreleg. Thin basophilic tip of regenerating muscle fiber arising from normal fiber and extending into area of necrosis containing many histiocytes. Of note are conspicuous proliferation of muscle nuclei and irregular scalloped end of fiber. Hematoxylin and eosin stain. × 450.

FIG. 8. Pectoralis major. Thin bands of regenerating muscle arise from fiber on left and extend around central core of necrotic débris. Of note are the proliferation of muscle nuclei, the basophilic cytoplasm, and absence of cross striations in the regenerating band of muscle. Hematoxylin and eosin stain. × 600.

FIG. 9. Muscle from right anterior thigh, showing proliferation of muscle nuclei within a regenerating fiber. Striations are absent in central portion of fiber, and the fiber is small in comparison with the one adjacent. Hematoxylin and eosin stain. × 810.



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VARICELLA: REPORT OF TWO FATAL CASES WITH NECROPSY, VIRUS ISOLATION, AND SEROLOGIC STUDIES*

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Although much has been learned regarding the pathogenesis of varicella, further progress in the understanding of this disease and its relation to herpes zoster has awaited the development of methods for the isolation and propagation of the etiologic agents. Such methods have been devised recently in this laboratory.[1,2] They depend essentially upon the capacity of the virus to produce characteristic focal lesions in cultures of human tissues inoculated either with material from the cutaneous eruption of varicella or from cultures in which the agent has previously multiplied. Within the affected cells comprising the focal lesions, acidophilic intranuclear inclusions are observed regularly. The focal lesions produced by the varicella virus are indistinguishable from those observed following the inoculation of similar cultures with material from the cutaneous lesions of patients with herpes zoster. As a result of the isolation *in vitro* of the agents, immunologic procedures, such as the fluorescent antibody technique[3] and the complement fixation test,[4] could be applied to the investigation of these diseases. In this paper are described studies of two fatal cases of varicella in which these techniques were employed together with the pertinent clinical and post-mortem findings.

REPORT OF CASES

Case 1

P. C. (no. 407338) was a 4-year-old white boy who was first seen in the Children's Medical Center in November, 1953; a neuroblastoma of the left adrenal gland was removed at that time, together with the left kidney, spleen, and the tail of the pancreas which was infiltrated by tumor. X-ray irradiation totaling 2,440 r. (calculated tumor dose) to the left hemi-abdomen was completed on November 24. Evidence of metastasis to the right humerus was found on December 30 and the patient was referred to the tumor therapy clinic of the Children's Cancer Foundation. Daily administration of 5 mg. of 4-amino-N-10-methyl pteroylglutamic acid (methotrexate) was started immediately. On January 19, following the appearance of uncomplicated varicella in three siblings, a single vesicle appeared on his face.

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Additional crops of vesicles appeared over the next several days, involving all parts of the skin and accompanied by lesions in the mouth. Aureomycin therapy was started on January 21. On January 25, the patient complained of soreness of the mouth and throat and on January 27, because of poor oral intake, was admitted to the hospital with varicella of 8 days' duration.

On admission the child was poorly nourished and acutely ill; temperature, 103.6°F.; pulse, 136; respirations, 28; blood pressure, 82/42 mm. of Hg. An almost monomorphous vesicular rash covered the skin, presenting an appearance closely resembling that of variola. Intact or ruptured vesicles also were present on the nasal, buccal, lingual, and genital mucosae, on the conjunctivae, and in the external auditory canals. Inguinal and axillary nodes were slightly enlarged. The extremities were painful to palpation at the sites of known or suspected metastases. Otherwise, the findings on physical examination, including that of the lungs, were not remarkable.

Throughout the 9-day period of hospitalization the patient was lethargic and his temperature varied between 99° and 101° F. Smears made from the vesicles showed giant cells of the type produced by the viruses of herpes simplex, varicella, and herpes zoster.[5] Methotrexate therapy was discontinued on the third hospital day. Many of the vesicles ruptured, exuding serosanguineous fluid, and a few new ones appeared. Although the contents of some of the vesicles became turbid, the vesicles failed to become purulent. The white blood cell count rose from 5,900 (77 per cent polymorphonuclear cells, 22 per cent lymphocytes, and 1 per cent eosinophils) on admission to 14,400 on February 1. Diarrhea developed and aureomycin therapy was replaced by penicillin and erythromycin. Stool culture at that time yielded *Monilia stellatoidea*. On February 2, 10 cc. of gamma globulin were given. The appearance of the patient at this time is shown in Figure 1. On February 3, blood culture yielded *Pseudomonas aeruginosa*. On February 4, 9 hours prior to death, the patient developed signs of peripheral circulatory collapse; the lungs became filled with coarse râles and petechiae developed between the vesicles. The total white blood cell count was 4,800. Lumbar puncture revealed no abnormalities. Death occurred 17 days after appearance of the first vesicle.

Necropsy was begun 2½ hours after death. Tissues were fixed in Zenker's fluid, except the brain and spinal cord which were fixed in formalin; hematoxylin and eosin stains were used.

The skin had an extensive vesicular eruption, of maximal prominence on the face and back, and minimal on the hands and feet. Most of the lesions were 0.5 to 1.5 cm. in diameter with indurated, hemorrhagic bases. Some contained turbid fluid but many had ruptured and were covered with serosanguineous fluid or crusts. In addition, there were scattered, smaller, light red to violaceous, vesiculo-papular lesions, some of which contained clear fluid. On the right side of the abdomen and back and involving the dermatome of the tenth thoracic nerve was a band of confluent vesicles which extended within 3 to 4 cm. of the midline both anteriorly and posteriorly.

Microscopically, the early cutaneous lesions consisted of minute unilocular vesicles in the malpighian layer of the epidermis; the epithelial cells within and bordering such vesicles contained moderately basophilic homogeneous inclusion bodies which filled the nuclei (Figs. 2 and 3). The endothelium of capillaries adjacent to these

lesions frequently contained intranuclear inclusions. Similar inclusions were seen in fibroblasts bordering affected vessels. The larger cutaneous lesions also were essentially unilocular. In these, the intranuclear inclusions observable in a variety of cell types were generally more acidophilic and were separated from the marginated layer of nuclear chromatin by a clear halo. Multinucleated giant cells containing inclusions were seen within the vesicles. In several instances there was destruction of the germinal layer with necrosis and hemorrhage in the superficial dermis. Small areas of necrosis were present in the hair follicles and adnexal glands. Numerous vessels were necrotic and surrounded by polymorphonuclear leukocytes; no inflammatory infiltrate was seen within the epidermal vesicles. No alteration of the cutaneous nerves was noted, either in the area of the zosteriform eruption or of the disseminated vesicles.

There was marked facial edema, particularly of the periorbital tissues. The conjunctivae were congested and showed vesicular lesions. Many vesicular and ulcerated lesions were seen on the nasal, buccal, and lingual mucosae. Sections taken from the tongue showed superficial ulceration with necrosis, secondary bacterial invasion, and acute inflammatory infiltrate in the exposed tissues. Intranuclear inclusions were observed in the superficial and glandular epithelium, fibroblasts, and vascular endothelium.

There was no increase in fluid in any of the body cavities.

The thymus was atrophic. Rare focal areas showed acidophilic intranuclear inclusions within what appeared to be endothelial and reticulum cells. Lymph nodes showed moderate to marked lymphoid depletion.

The heart and major vessels were not remarkable grossly and the only microscopic finding of note in respect to them was the presence of a few lymphocytes within the myocardium.

The lungs were congested, with the right weighing 166 gm. and the left 136 gm. On external and cut surfaces were scattered, firm, dark red areas of consolidation 0.5 to 1.2 cm. in diameter. The trachea and bronchi contained a large amount of mucus. Microscopically, protein-rich edema fluid was evident within the alveolar lumina. In areas of consolidation there was extreme capillary congestion and alveolar hemorrhage with autolytic necrosis in the central portion of the lesions (Figs. 4 and 5). In the peripheral areas of the consolidated lesions, a few amphophilic to acidophilic intranuclear inclusion bodies were seen in alveolar lining cells, alveolar macrophages, bronchial and bronchiolar epithelium, fibroblasts, and endothelial cells.

At the periphery of these lesions there was minimal inflammatory infiltration, consisting chiefly of polymorphonuclear leukocytes. There was gangrenous tracheitis showing bacteria, and there were intranuclear inclusions in epithelial and other cells.

Many papular, vesicular, and ulcerative lesions measuring up to 1 cm. in diameter were present in the mucosa of all parts of the alimentary tract except the esophagus. Microscopically, the many ulcers gave evidence of three etiologic agents. In some there was bacterial invasion of the denuded tissues and a moderate amount of acute inflammatory infiltrate. In others hyphal and budding yeast forms were seen in large numbers and usually these were associated with little cellular reaction. Lastly, in some of the ulcers acidophilic intranuclear inclusion bodies were seen; these inclusions usually were few but involved endothelium, fibroblasts and, rarely, mucosal epithelium. Little cellular infiltrate was noted in these lesions except where bacteria were present also. In numerous sections of small intestine and even in the absence of local ulceration, typical inclusion bodies were seen in cells of the myenteric plexus. Smooth muscle cells and fibroblasts adjacent to affected nerves frequently contained similar inclusions, but no cellular infiltrate was present.

The liver was slightly enlarged and congested, weighing 610 gm. Scattered over the external surface were dark red vesicular lesions, 0.2 to 0.4 cm. in diameter. Throughout the substance of the liver were dark red areas and yellow opacities 0.2 to 0.3 cm. in diameter, distributed without reference to the lobular pattern. Microscopically, these corresponded to areas of necrosis in which there was little neutrophilic infiltration. In such foci occasional intranuclear inclusions were seen within endothelium, Kupffer cells, fibroblasts, and parenchymal cells (Fig. 6). The periportal areas were diffusely infiltrated with lymphocytes and other mononuclear cells.

The gallbladder and biliary passages showed no gross lesions. The spleen, left adrenal gland, left kidney, and tail of the pancreas were absent. The remaining pancreatic tissue was not remarkable grossly. The adjacent tissues showed old fat necrosis and recurrent tumor. Microscopically, the pancreas had small areas of necrosis with intranuclear inclusion bodies within acinar cells, endothelium, and fibroblasts (Fig. 7).

The right adrenal gland was not remarkable grossly; microscopically, focal lesions within the medulla contained intranuclear inclusions.

The right kidney was slightly enlarged, weighing 75 gm. On its external surface were numerous raised, red, vesicular lesions 0.2 to 0.3 cm. in diameter, and on section dark red, firm nodules of similar size were seen in both cortex and medulla. The renal pelvis showed focal areas of hyperemia and an occasional petechia. The general architecture of the kidney was well preserved. There were small foci of necrosis microscopically, with associated hemorrhage but with little cellular infiltration. In these lesions, inclusion bodies were seen within endothelium, fibroblasts, cells of the glomerular tuft, and convoluted tubules. Several microscopic tumor metastases were seen in the cortex. No inclusions could be identified within either pelvic or bladder mucosa.

The testes showed a few petechiae. Microscopically, intranuclear inclusions were seen in the endothelium of capillaries and small arteries associated with these hemorrhages and in adjacent fibroblasts. Inclusions were seen also within cells of a single seminiferous tubule.

Sections of the right humerus and the body of the first lumbar vertebra showed extensive infiltration of the marrow spaces by neuroblastoma. Tumor was seen also in numerous lymph nodes. In all these locations there was necrosis of the neoplasm, with intranuclear inclusions in occasional tumor cells as well as in the endothelial and connective tissue elements.

Sections of submaxillary gland showed foci of necrosis with intranuclear inclusions in ductal and acinar cells.

No gross or microscopic lesions of the thyroid gland were seen.

No gross alterations of the dorsal root ganglia, spinal cord, or brain were noted. The dorsal root ganglia on the right of T-6, T-9, T-10, and T-11 were examined microscopically (Fig. 8). These showed acidophilic intranuclear inclusions in many satellite cells. Such inclusions were few in the ganglion thought to supply the area of zoster eruption, that is, T-10, but were numerous in other ganglia. Although numerous ganglion cells showed degenerative changes with peripheral dispersion of Nissl substance, nuclear pyknosis, and karyolysis, only rare ganglion cells contained inclusions. These were seen in T-6 and T-10 and were large, irregular basophilic bodies which were irregularly separated from the nuclear membrane by a clear halo. In one section of spinal cord, probably from the lower thoracic region, there was minimal perivascular lymphocytic infiltration in the posterior columns and anterior horns. No inclusion bodies were seen in the cord and no microscopic lesions of the brain were noted.

Case 2

L. O. (no. 9244) was a 7-year-old white girl who was admitted to the House of the Good Samaritan Hospital in August, 1954, with a febrile illness of 24 days' duration. Physical and laboratory findings were consistent with a diagnosis of acute rheumatic fever with mitral insufficiency and active carditis. Daily administration of 300 mg. of cortisone was followed by clinical improvement. Four weeks after institution of this therapy and 18 days after exposure to a patient with varicella, a pleomorphic vesicular rash appeared on her trunk, back, and face. At the same time she began to complain intermittently of abdominal pain. Temperature remained normal. There was generalized abdominal tenderness, but peristaltic sounds were not unusual. Blood pressure, which had not been elevated previously, rose to 140/90-110 mm. of Hg. The white blood cell count was 21,000 with 50 per cent segmented neutrophils, 12 per cent band cells, 28 per cent lymphocytes, 8 per cent monocytes, and 2 per cent eosinophils. Abdominal pain continued and on the third day of her rash she began to pass bloody stools. Bleeding and clotting times were normal and platelets appeared normal on smear. Coma, cyanosis, and muscular twitchings developed and were followed by generalized convulsions even though the administration of oxygen under positive pressure relieved the cyanosis. Signs of shock developed but disappeared on treatment. Lumbar puncture revealed no abnormalities. She vomited bloody fluid and blood was obtained by endotracheal suctioning; massive bloody stools continued. She expired on the fourth day of her rash, 12 hours after the onset of coma.

Necropsy was begun 7 hours after death. Tissues, including spinal cord and representative areas of brain, were fixed in Zenker's fluid; the remainder of the brain was fixed in formalin. Sections were stained with toluidin blue and eosin.

The body was well developed and well nourished. There was definite hirsutism and a typical Cushing's facies. The conjunctivae were without lesions. Blood was exuding from the nose and mouth and several small vesicles were seen in the buccal mucosa. The abdomen was slightly distended. There was clubbing of the fingers and toes. External lymph nodes were not enlarged. A maculo-papular to vesicular rash involved the skin of the head, neck, trunk, and thighs. The individual lesions averaged 1 to 2 mm. in diameter. Some of these were slightly hemorrhagic and on cut surface the hemorrhage extended approximately 1 mm. into the subcutaneous fat in a triangular wedge.

Microscopically, the skin lesions resembled those of case 1. However, they generally represented the earlier stages in the development of the lesion of varicella, and differed in showing a moderate leukocytic infiltrate.

The serosa of the peritoneal, pleural, and pericardial cavities showed a few petechiae. There was a slight increase in fluid within all body cavities, with that in the left pleural cavity serosanguineous.

Lymph nodes and the thymus were quite small, the latter weighing 9 gm. They showed marked lymphoid depletion, swelling of vascular endothelium, and focal capillary congestion. Hassall's corpuscles were

filled with keratinized débris and showed extensive necrosis, with only a few peripheral cells remaining. Many of the latter contained acidophilic intranuclear inclusions. Intranuclear inclusions were very numerous within reticular cells of the thymus and occasionally were seen within thymic lymphocytes.

The heart weighed 165 gm. The myocardium was flabby and there was moderate dilatation of all chambers. The endocardium of the left ventricle and atrium was thickened, as were the cusps and chordae tendineae of the mitral valve.

Microscopically, there was marked edema of all layers of the heart. Focal capillary congestion and occasional hemorrhages were seen. Vascular endothelium was swollen and, although the nuclei were vesicular, none contained inclusion bodies. No fibrinoid necrosis or typical Aschoff bodies were seen. However, there were numerous small cellular accumulations in the adventitia of small vessels, particularly in the myocardium. Some of these were of fibroblasts while others contained large mononuclear cells and lymphocytes. In areas there was a more diffuse infiltration of the myocardium with lymphocytes and polymorphonuclear leukocytes.

The lungs weighed 556 gm. They maintained their shape to an abnormal degree after removal and were subcrepitant and of increased consistency. The lungs were dark purple; the pleural surface showed numerous petechiae, a few tiny hemorrhagic vesicles, and darker red, elevated areas which corresponded to the lobular pattern (Fig. 9). The latter were found to be hemorrhagic areas of consolidation measuring up to 2 or 3 cm. in diameter.

Microscopically, there was extreme congestion throughout the lungs and large areas of intra-alveolar hemorrhage (Fig. 10). Macrophages were present in large numbers within the alveolar lumina. A few lymphocytes and polymorphonuclear leukocytes were present in the alveoli and in interstitial tissues. Intranuclear inclusions were seen within occasional alveolar macrophages and fibroblasts, and were noted also in tracheal epithelium. Endothelial cells were swollen but none contained inclusion bodies.

The spleen was slightly enlarged, weighing 77 gm. Verrucal capsular thickening and occasional vesicles were seen along the medial edge. The organ was congested and showed many gray opacities 1 to 2 mm. in diameter throughout its substance. Microscopically, these were seen to be foci of necrosis involving the red pulp and to a less extent septa and the small malpighian bodies. In these focal lesions, intranuclear inclusions occurred in fibroblasts, smooth muscle, and sinusoidal endothelium, and were very numerous also in the swollen

sinusoidal cells throughout the organ. There was little leukocytic response around the areas of necrosis.

In the lower third of the esophagus were several confluent mucosal hemorrhages. Throughout the stomach and the small and large intestines, the mucosa was extremely congested and hemorrhagic and the intestinal lumen was distended with bloody fluid. Sections of the esophagus showed several groups of epithelial cells with ballooning degeneration and acidophilic intranuclear inclusion bodies. Lesions in other portions of the gastro-intestinal tract were limited to congestion and hemorrhage.

The pancreas showed no gross or microscopic lesions.

The liver was enlarged, weighing 930 gm. There were numerous subcapsular hemorrhages. On the cut surface there were seen numerous dark red opacities measuring up to 2 mm. in diameter. Microscopically, there were foci of necrosis similar to those of case 1 but showing slightly more leukocytic infiltration. Inclusion bodies were rare but occurred within cells of the same types as in case 1.

The adrenal glands were small and their cortices thin and atrophic. One adrenal gland showed intranuclear inclusions in several medullary cells. Intranuclear inclusions were seen within cells of the theca interna of developing ovarian follicles. No other gross or microscopic lesions were seen in the kidneys, bladder, internal genitalia, or thyroid gland.

There was hemorrhage along the right sympathetic chain of ganglia but no specific lesions were associated. The brain and spinal cord showed no lesions. No inclusion bodies were seen in dorsal root ganglia which were examined from several levels. The pituitary body showed numerous degranulated basophils.

Vertebral and costal marrow showed a slight decrease in cellularity, chiefly in the myeloid series. No maturation arrest was present. Endothelial and stromal reticular cells contained intranuclear acidophilic inclusions.

Laboratory Investigations

Virus Isolation and Propagation

Roller tube cultures of human tissues were employed.[2,6] Cultures of human embryonic skin-muscle tissue were utilized for isolation; passages were carried out thereafter either in this tissue or in cultures of human foreskin. The beef amniotic fluid medium of Enders[7] (90 per cent beef amniotic fluid, 5 per cent beef embryo extract, and 5 per cent horse serum) in 2 ml. amounts was utilized routinely in each culture. Penicillin (100 units per ml.) and streptomycin (100

μgm. per ml.) were incorporated in the medium and also were employed in the preparation of suspensions of tissues. Passages *in vitro* were carried out by the transfer of aliquots of finely ground infected tissue (generally 0.1 ml. per culture) as previously described.[2] Ground tissues from uninfected cultures were passed similarly in series.

Materials employed as inocula from case 1 were: (a) vesicle fluid collected in buffered skimmed milk on the eleventh day of illness (frozen in sealed glass ampules in the CO_2 cabinet for 94 days prior to inoculation); (b) blood collected from the right atrium at necropsy and prepared for immediate inoculation as a 10 per cent suspension by grinding the clot in isotonic phosphate buffer (pH, 7.1 to 7.2); (c) portions of lung, adrenal gland, right axillary lymph node, kidney, and liver (10 per cent suspensions in phosphate buffer). Cultures were inoculated with 0.1 ml. of the suspensions of lung or adrenal gland within approximately 11 hours of death. The suspensions of lymph node, kidney, and liver were stored in the CO_2 cabinet for 3 weeks prior to inoculation. In each instance three tissue cultures were inoculated.

Cultures receiving suspensions of adrenal gland and lymph node became contaminated with bacteria and were discarded. Those inoculated with liver and kidney showed no specific cytopathic changes during an observation period of 52 days. By the eighth day after inoculation, two to four foci of degeneration had developed in each of the three roller tubes inoculated with the suspension of lung tissue. The foci were similar to those described previously[2] and consisted of collections of rounded, swollen, refractile cells which slowly increased both in size and number. Similar changes occurred in one of the three cultures inoculated with blood but were not apparent until 20 days after inoculation. All three tubes receiving vesicle fluid developed specific changes by the eighth day after inoculation.

From case 2, vesicle fluid and blood were collected at necropsy. Eleven hours after death, three cultures were inoculated with 0.1 ml. amounts of the vesicle fluid which had previously been mixed with a small quantity of sterile neutralized milk. To each of three other cultures 0.2 ml. of undiluted blood was added. On the seventh day after inoculation one tube receiving vesicle fluid exhibited focal cytopathic changes similar to those produced by materials from the previous case and all three were affected by the eleventh day. The three cultures inoculated with blood all contained focal areas of degeneration by the fifth day and thereafter additional foci developed with unusual rapidity.

Passage of Agents Isolated

Case 1. Passage of the agent isolated from vesicle fluid of case 1 was not attempted. The agent isolated from the blood was carried through four passages in cultures of embryonic skin-muscle; this strain was discontinued after being maintained *in vitro* for a period of 147 days. The virus isolated from the lung was carried through 14 passages for an elapsed period of 492 days utilizing cultures of either human embryonic skin-muscle or foreskin.

Case 2. The agents isolated from vesicle fluid and blood of case 2 were each maintained for four passages in embryonic skin-muscle tissue of foreskin over a period of 78 days. The strain isolated from blood has been carried subsequently through three additional passages for 84 days by Dr. John Bell.

In each instance parallel control cultures failed to show specific degeneration of the type herein described.

Identification of Agents Isolated

Tissue culture preparations infected with the agents isolated from the lung and blood of case 1 and that from the blood of case 2 were fixed in Bouin's or Zenker's fluid and stained with hematoxylin and eosin. Cells in and bordering the foci of degeneration contained type A acidophilic intranuclear inclusions (Fig. 11). In some instances infected cells had multiple nuclei, each of which showed an inclusion.

Strains of tissue culture virus from each patient were inoculated intracerebrally and intraperitoneally into 1-day-old white mice of the Harvard strain without producing clinical disease. The agent obtained from the blood of case 1 was inoculated also onto the chorioallantois of 10-day-old chicken embryos without producing grossly recognizable lesions.

Additional evidence indicating that the agent isolated from the lung of case 1 was that of varicella was obtained by means of the fluorescent antibody technique.[8] Tissue infected *in vitro* with this virus (Cic strain) showed no fluorescence in the presence of serum collected during the acute phase from a known case of varicella and intense fluorescence in the presence of serum collected during the convalescent phase.

Serologic Studies

Serum specimens obtained on the twelfth day of illness and also post mortem from case 1 were negative in a dilution of 1:8 when examined by the complement fixation method. A post-mortem blood

specimen from case 2 likewise contained no demonstrable complement-fixing antibody in the presence of varicella antigen when examined in a 1:4 dilution. In these tests the antigen was prepared from tissue culture materials infected with a known strain of varicella virus; previously this antigen had been found to react consistently with sera from the convalescent phase of cases of varicella.[4]

Discussion

The pathologic changes observed at necropsy in both cases resemble, in general, those previously reported in fatal cases of varicella.[8-13] The lesions consist of focal areas of necrosis, frequently containing type A intranuclear inclusions, and may be found in many organs, particularly in the skin, liver, lung, spleen, adrenal glands, gastrointestinal tract, and pancreas. The inclusions may be seen in cells of various types. However, it is of particular interest in considering the pathogenesis of this disease, that in the classical description of the pathologic response in the skin by Tyzzer[14] the occurrence of intranuclear inclusions in vascular endothelium was noted very early in the evolution of the lesions. Similar involvement of endothelium in internal organs has been mentioned specifically in certain of the published necropsy reports[8,12] and was seen in both cases herein described.

Morphologic differentiation of the disseminated lesions of varicella from those occasionally seen in the course of herpes zoster would appear to be impossible.[15,16]

Each of the cases reported here has morphologic features of particular interest. Case 1 presented a clinical picture consistent with severe chickenpox but had at necropsy a typical zosteriform eruption in addition to the disseminated varicella lesions. The dorsal root ganglion supplying the affected dermatome, as well as three other ganglia examined, showed intranuclear inclusions. Neural involvement was seen also in the small intestine, where there were many inclusions in cells of the myenteric plexus. Although involvement of the dorsal root ganglia may be a result of viremia, the simultaneous involvement of these ganglia and the myenteric plexus is also consistent with a neurogenic spread of the virus.

In case 1 varicella was of unusual chronicity, apparently without a significant immune response. Whereas in the average case of varicella complement fixing antibody appears about the fifth day of illness and rapidly increases to high levels,[4] in case 1 no such response was demonstrable and the patient continued to develop vesicles over a

period of 17 days. At death, virus was recovered from the blood. The comparative absence of a cellular inflammatory response in the fully developed necrotic lesions also was striking.

It is of interest that gamma globulin determinations done on the first patient (case 1) by Dr. David Gitlin revealed no evidence of hypogamma-globulinemia. It is to be remembered that this patient had disseminated neuroblastoma treated with radiation and methotrexate. The significance of these factors in the genesis of the unusual response to varicella virus is obscure at present.

The striking morphologic feature of case 2 was the severity of the vascular damage, with edema and widespread hemorrhage. The vascular endothelial cells were swollen in most organs, though inclusion bodies were seen only occasionally within them. Extensive and severe vascular involvement was observed in the sinusoids of the spleen where inclusion bodies were numerous. The focal hemorrhages were associated with definite evidence of local viral infection. The more diffuse endothelial damage may represent a stage of the same process. In view of the frequent association of severe cases of chickenpox with leukemia, the identification of inclusion bodies within what appeared to be lymphocytes is of particular interest.

In contrast to the first patient, who had a relatively chronic infection, the second died after a fulminating illness of short duration. Because of the short duration, the absence of complement-fixing antibodies at the time of death is not unexpected. Post-mortem determinations by Dr. David Gitlin revealed normal concentrations of gamma globulin in the blood, though it is to be noted that this patient received several transfusions immediately prior to death. Again, virus was recovered from the blood. This patient had rheumatic fever and was under treatment with cortisone. There has accumulated sufficient evidence with other viral agents[17] to suggest that in this instance cortisone may have influenced the severity of the infection. Further suggestion that cortisone may unfavorably influence the course of varicella is found in a recent report of a single patient who, while on cortisone therapy, had a recurrence of varicella 1 month after the initial attack.[18] We have recently seen a second rapidly fatal case of varicella in a patient under cortisone therapy; in this patient, as in the second patient of this report, circulatory collapse was a prominent clinical feature.

Summary

Two fatal cases of varicella are reported, with necropsy findings and viral studies.

The first was a 4-year-old boy who, while under therapy for dis-

seminated neuroblastoma with 4-amino-N-10-methyl pteroylglutamic acid, developed varicella with vesicles continuing to appear for 17 days. Morphologic features of varicella in this patient were remarkable chiefly for a diminished inflammatory response, a zosteriform eruption, and evidence of viral infection of multiple dorsal root ganglia and the myenteric plexus of the small intestine. Virus was isolated from the blood, vesicle fluid, and lung by tissue culture techniques. In spite of the duration of the disease, demonstrable specific complement-fixing antibodies did not appear.

The second case occurred in a patient with acute rheumatic fever under therapy with cortisone. It was distinguished by a more fulminating course with widespread vascular damage and hemorrhage demonstrable at necropsy. Varicella virus was recovered also from the blood and vesicle fluid of this patient.

We are indebted to Mr. John Carabitses for the preparation of the photomicrographs and Figure 1.

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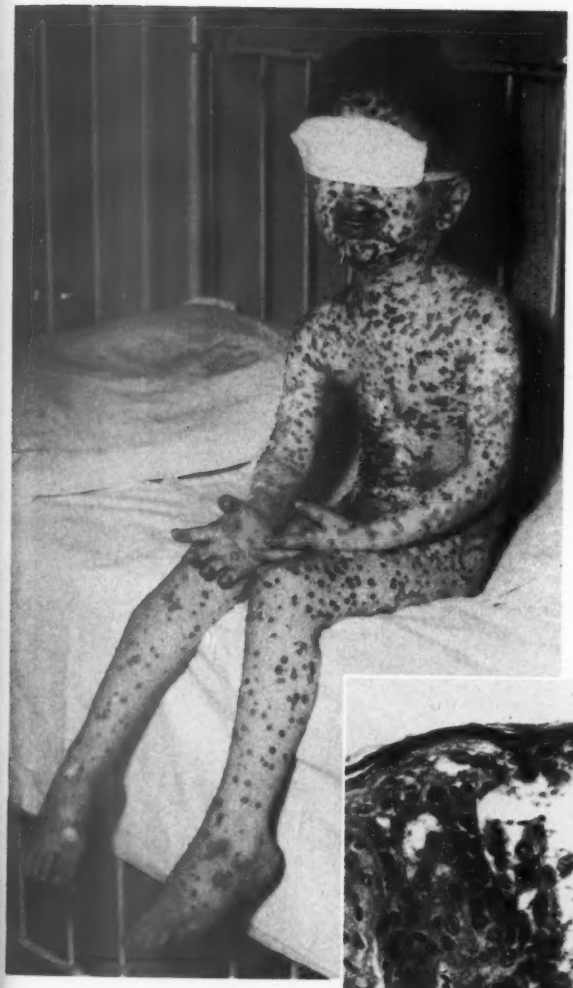
LEGENDS FOR FIGURES

FIG. 1. Case 1. Appearance of patient on the 15th day of exanthem.

FIG. 2. Case 1. Immature cutaneous vesicle present on 17th day of exanthem. × 260.



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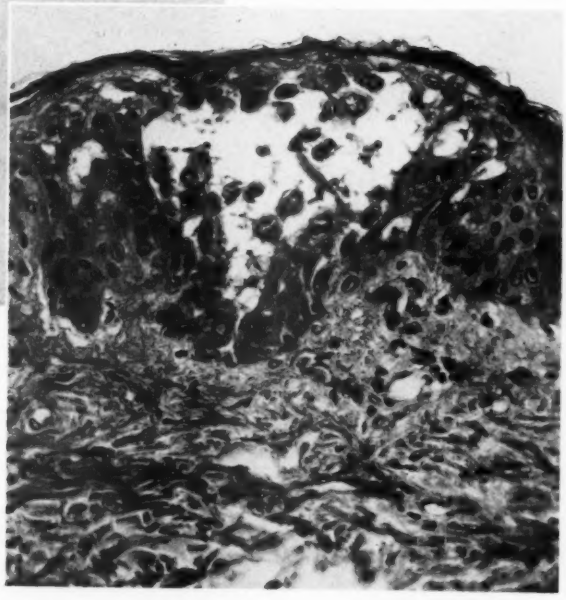

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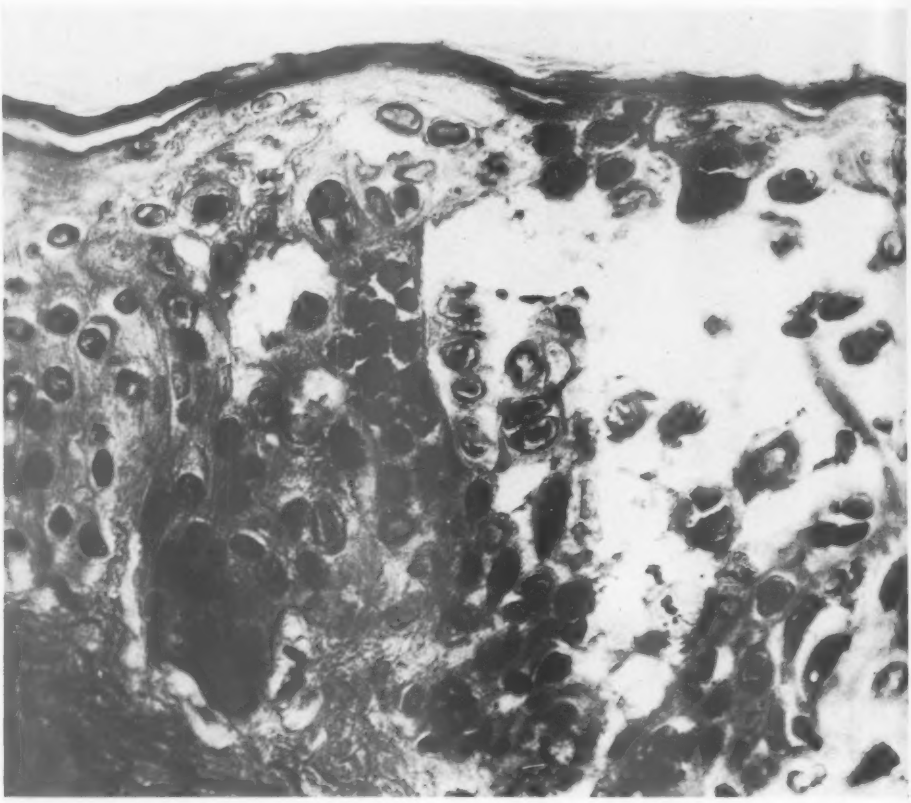
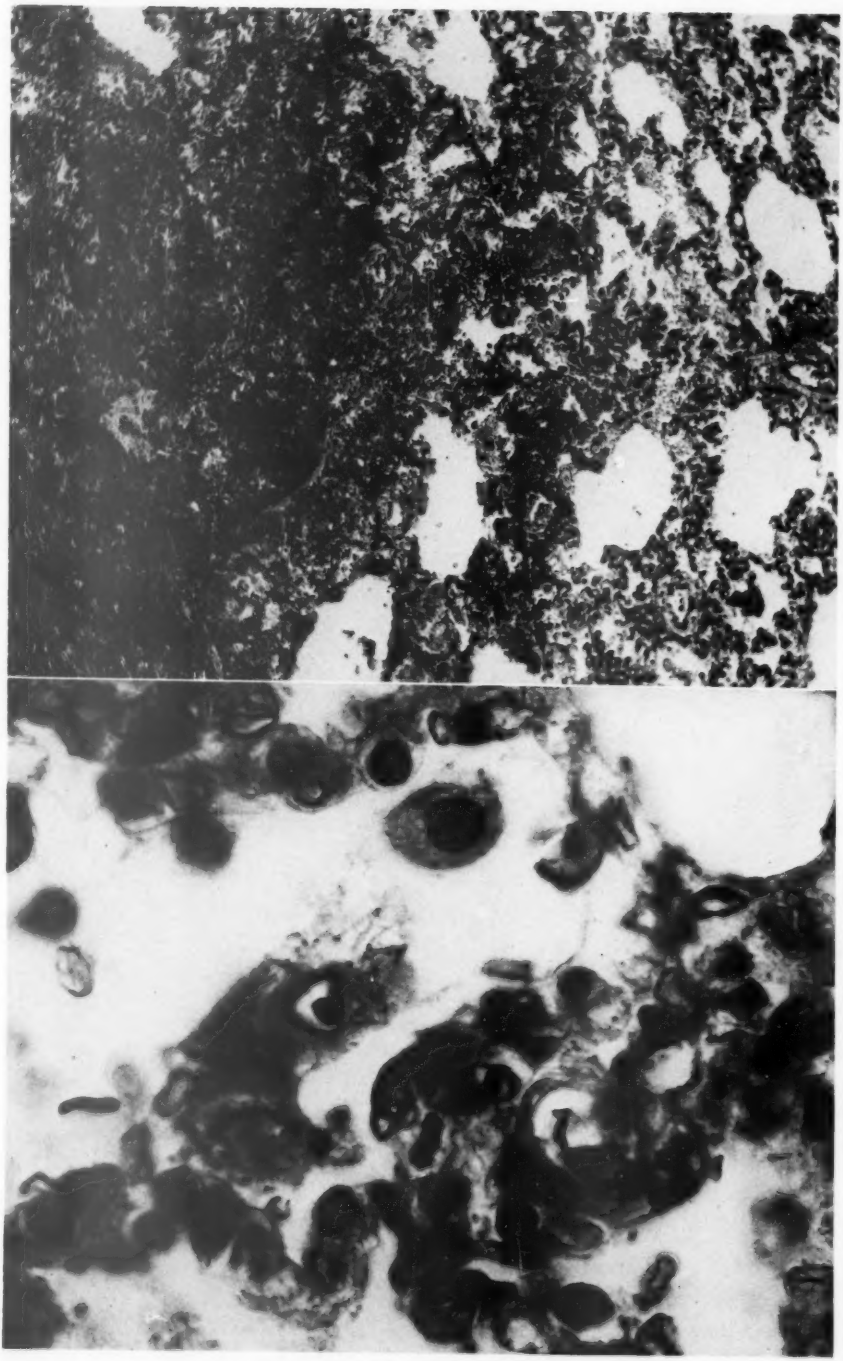


FIG. 3. Case 1. Margin of vesicle pictured in Figure 2 showing numerous intranuclear inclusions. × 645.

FIG. 4. Case 1. Lung showing margin of an area of hemorrhagic consolidation. × 120.

FIG. 5. Case 1. Pulmonary alveoli showing intranuclear inclusions. × 1230.



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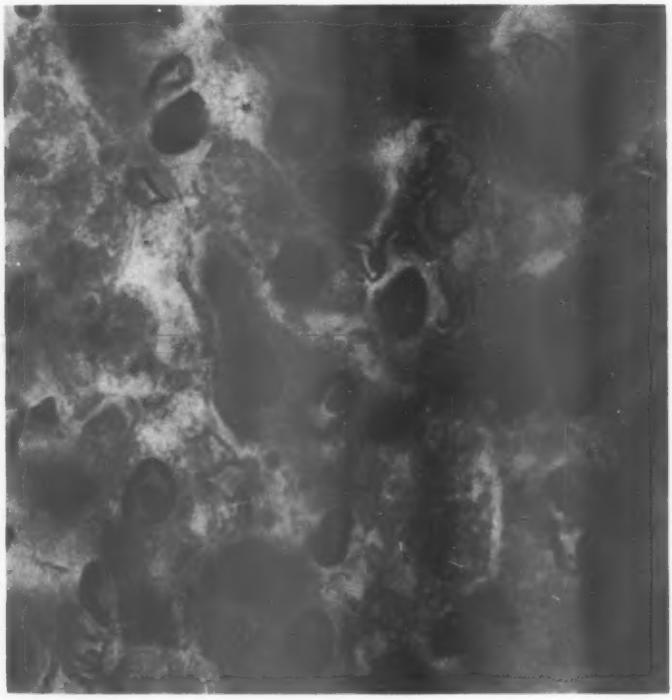
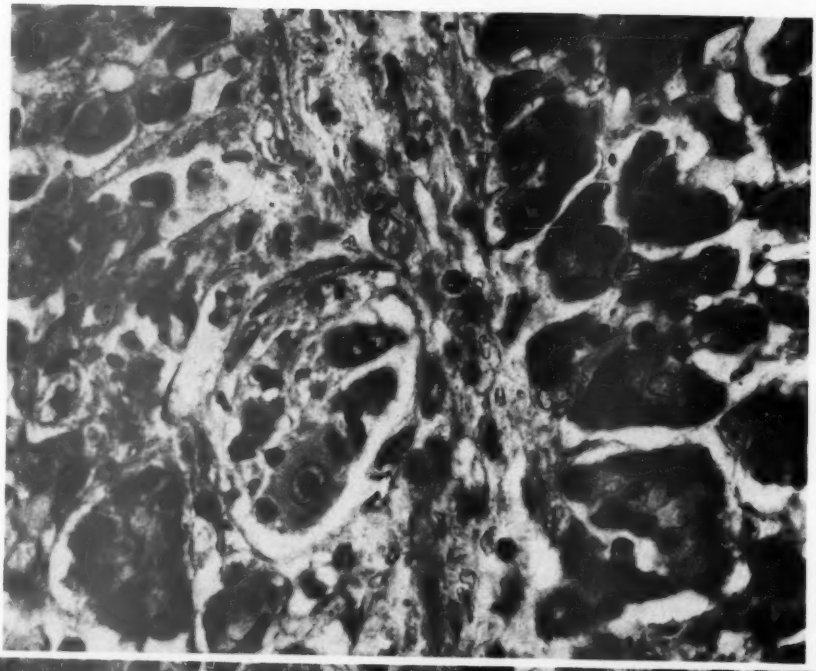


FIG. 6. Case 1. Intranuclear inclusion in liver cell. × 1400.

FIG. 7. Case 1. Edge of focus of necrosis in pancreas with inclusions in parenchymal cells. × 600.

FIG. 8. Case 1. Right dorsal root ganglion (T-6) showing typical intranuclear inclusions in satellite cells and basophilic body in nucleus of ganglion cell. × 600.



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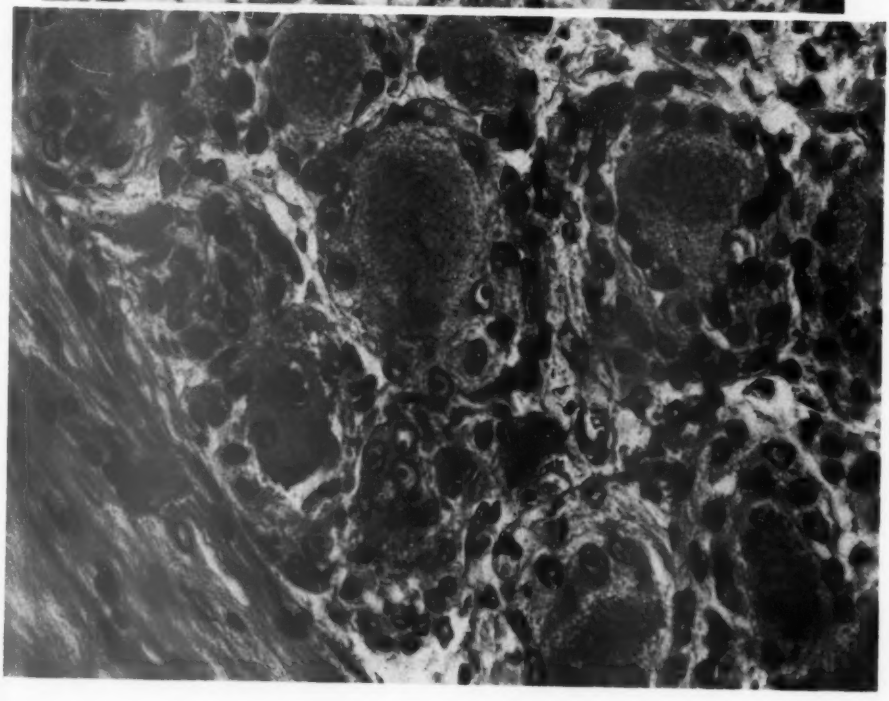

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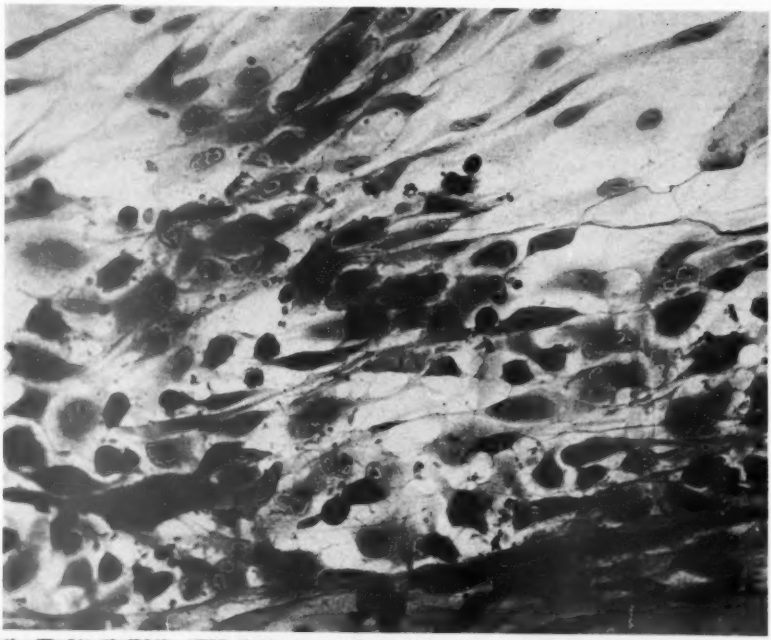
FIG. 9. Case 2. Lungs.

FIG. 10. Case 2. Lung showing congestion, edema, and minimal inflammatory infiltrate. × 150.

FIG. 11. Second tissue culture passage of agent isolated from blood of case 1. Coverslip preparation stained with hematoxylin and eosin, showing characteristic intranuclear inclusions. Human embryonic skin-muscle tissue 13 days after inoculation. × 290.



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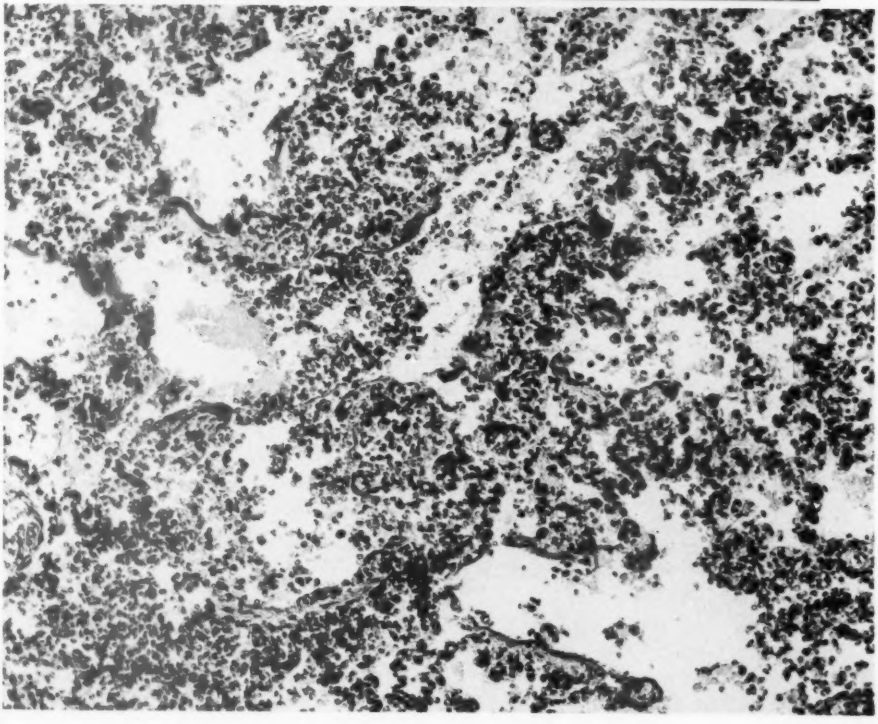

10

EXPERIMENTAL HYPERTENSIVE VASCULAR DISEASE ACCOMPANYING ADRENAL REGENERATION IN THE RAT*

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In 1946, Selye[1] first enunciated his concept that certain diseases in man such as hypertension, arteriolar nephrosclerosis, fibrinoid arteriolar necrosis, and periarteritis nodosa were the result of maladaptation to non-specific stress. Since a principal homeostatic mechanism initiated by exposure to non-specific stress is functional activation of the anterior-hypophyseal-adrenocortical system, Selye postulated that these diseases were the direct result of some pathologic aberration of this physiologic reaction. The exact nature of this abnormal function still remains obscure, but experiments in which rats were treated with desoxycorticosterone acetate (DCA)[2] or lyophilized anterior pituitary substance (LAP)[3] suggested that increased secretion by the adrenal cortex of mineralo-corticoids, such as desoxycorticosterone acetate, leads to their development. While certain recent evidence seems to support this broad hypothesis, one of the weak links in the chain of supporting evidence has always been that, with the single exception of cold, non-specific stress has been notably ineffective in producing experimental hypertension and vascular necrosis.[4]

It has been shown recently that young, unilaterally nephrectomized rats maintained on 1 per cent sodium chloride develop hypertension and widespread cardiovascular-renal lesions following adrenal-enucleation.[5] The hypertension and vascular changes develop as the adrenal cortex regenerates. This observation represents strong evidence that abnormal adrenal cortical function may participate in the genesis of at least certain forms of hypertension and vascular disease. The lesions associated with this "adrenal-regeneration hypertension" of the rat closely resemble those of malignant hypertension in the human being and are described in detail in the present paper.

METHODS

Twenty-four female, unilaterally nephrectomized rats of the Sprague-Dawley strain, averaging 62 gm. in weight, were maintained on Purina Laboratory Chow and 1 per cent sodium chloride drinking

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fluid. Twelve rats served as controls (group I). Right-sided adrenalectomy and left-sided adrenal-enucleation[6] were performed on the remaining 12 rats (group II). In this procedure the capsule of the adrenal glands was incised and the glandular tissue extruded by the gentle application of pressure with curved forceps. Thus the medulla and most of the cortex was removed, leaving only a few cells of the zona glomerulosa attached to the capsule, which remained *in situ*.

During the experiment, saline consumption was measured daily and systolic blood pressure was determined weekly by the microphonic manometer method of Friedman and Freed.[7] Six rats from each group were killed at the end of 7 weeks and the remaining rats in each group at 14 weeks. Blood was collected at both intervals, and serum sodium and potassium were measured by the Beckman DU flame photometer and serum chloride was determined by the method of Schales and Schales.[8] Organs were weighed fresh on a metric balance and fixed in Zenker-formol solution, 10 per cent formalin, or both, for histologic preparations. All tissues examined were stained with hematoxylin and eosin. Frozen sections of the adrenal glands, kidney, heart, and liver were cut at 8 μ and stained with Sudan IV. Kidney, heart, adrenal glands, brain, pancreas, and mesenteric vessels were stained by the periodic acid-Schiff technique (PAS), and, except for the brain, by the McGregor modification of the Heidenhain-azan method. The brain was stained with phosphotungstic acid hematoxylin (PTAH). Adrenal glands were stained for bacteria by the Gram method, and kidneys were stained for iron by the Prussian blue reaction.

Results

Since the physiologic aspects of this experiment have been reported elsewhere,[5] a brief summary of these results will suffice here.

At no time did the saline consumption or serum electrolyte levels of the adrenal-enucleated rats differ significantly from the control values. Despite this, the systolic blood pressure of the adrenal-enucleated rats killed after 7 weeks was 217 ± 9 mm. of Hg and in those killed after 14 weeks it was 197 ± 16 mm. of Hg, compared to control values of 151 ± 6 mm. of Hg and 145 ± 7 mm. of Hg, respectively. The adrenal-enucleated rats developed renal, cardiac, and cerebral enlargement and thymic atrophy at 7 weeks, and the adrenal glands had regenerated to the same relative weight as both adrenal glands of the controls. In the adrenal-enucleated rats killed after 14 weeks, renal and cardiac hypertrophy also were present, although less marked, but cerebral enlargement and thymic atrophy were not ob-

served. The regenerated adrenal glands were significantly smaller than both adrenal glands in the controls.

The severity of the lesions in the organs and tissues examined were graded from zero to 4 plus grossly and microscopically, and the results are summarized in Table I. A description of the lesions in the various organs follows.

TABLE I

Evaluation of Vascular Lesions in Rats with "Adrenal-Regeneration Hypertension"

Group	Time	Kidney	Heart	Brain	Adrenal gland	Pancreas and mesentery	Liver, spleen, thymus, and ovary
I Control	wks. 7	Trace	0	0	0	0	0
	14	Trace	0	0	0	0	0
II Adrenal-enucleated	7	+++	++	++	++	++	+
	14	++	+	Trace	+	+	0

Adrenal Glands. The adrenal glands of the control rats were normal. The regenerated organs at both 7 and 14 weeks were nodular and had a pale tan, semi-translucent appearance with focal red or hemorrhagic spots beneath the capsule. Microscopically, normal cortical zonation was restored, but the medullary region was replaced by fibrous connective tissue and focal calcium deposits. Adenoma-like nodules were present occasionally, either outside the capsule or within the cortex. The zona glomerulosa was narrow and composed of small cells with dark nuclei and scanty, dense cytoplasm. In contrast, the zona fasciculata and zona reticularis contained large cells with small nuclei and much foamy, lightly staining cytoplasm. While the zona glomerulosa of the adrenal glands of the control rats contained virtually no lipid (Fig. 1), the zona glomerulosa of the regenerated cortices was filled with sudanophilic material to the capsule (Fig. 2). In the adrenal glands of the control rats, the inner cortical zones contained the normal complement of lipid evenly distributed as fine droplets in the cytoplasm. In the regenerated adrenal glands, lipid was depleted slightly from these zones, and that which remained was present as coarse droplets or crescents frequently having a light central zone that gave the droplet a target appearance.

Capsular arterioles showed acute fibrinoid necrosis, and occasional vessels were surrounded by "fibrinoid or hyaline material" which was PAS-positive and stained blue with the McGregor stain. Such hyaline material frequently extended into the underlying areas of the adrenal

cortex where focal areas of necrosis were present (Fig. 3). In the cortical cells adjacent to these areas of necrosis, PAS-positive, intracytoplasmic "colloid" globules were observed (Fig. 4).

Kidney. Grossly, the kidneys of the control rats were normal at both 7 and 14 weeks. However, the PAS and McGregor stains demonstrated that at both intervals there were a few scattered glomeruli undergoing necrosis (Fig. 5). These glomeruli showed slight parietal epithelial cell proliferation, early breakdown of capillary tufts, and a few PAS-positive droplets in the glomerular space and proximal convoluted tubule. There was also slight thickening of the tubular basement membrane of the involved nephron. Arteriolar changes were notably absent. In general, such renal lesions in the control rats were slightly more frequent at 14 than at 7 weeks, but in each group they were scarce and of minimal severity.

In the rats bearing regenerated adrenal glands, the kidneys were enlarged and pale, with fine cortical scars and small, scattered, gray and red spots. Microscopically, the changes were severe and consisted of glomerulonecrosis and fibrosis with proliferation of epithelial cells, exudation of hyaline material into the glomerular space, and glomerular hemorrhage (Figs. 6 and 8). Tubules were dilated, contained hyaline casts, and frequently showed athrocytosis of hyaline droplets (Fig. 8). Considerable brown pigment which was positive to the Prussian blue reaction was observed in epithelial cells of tubules and glomeruli (Fig. 9). There was thickening of tubular basement membranes and proliferation of tubular epithelium. Arterioles showed changes which were of two basic types (Fig. 7): (1) fibrinoid necrosis characterized by medial degeneration, absence of elastic lamellae, and the presence of hyaline, PAS-positive material throughout the arteriolar wall; and (2) concentric hyperplastic thickening and fibrosis of the wall without fibrinoid change. Occasional arterioles had characteristics of both types and others showed hemorrhage into the wall and surrounding connective tissue. Renal arterioles undergoing early fibrinoid change occasionally had small PAS-positive globules in the media (Fig. 19). The Sudan stain demonstrated that involved glomeruli and arterioles contained lipid and that lipid droplets were present in tubular epithelial cells of some of the involved nephrons (Figs. 10 and 11). No stainable fat was present in any of the kidneys of the control rats.

Heart. There were no lesions of the myocardium or coronary vessels in the control rats sacrificed at either 7 or 14 weeks. Among the rats with regenerated adrenal glands, 5 of 6 showed lesions at 7 weeks and 2 of 5 at 14 weeks. The predominant vascular change

was acute fibrinoid necrosis accompanied by adventitial fibroblastic proliferation and accumulation of Anitschkow myocytes, lymphocytes, and occasional polymorphonuclear leukocytes (Figs. 12 and 13). Focal hyaline degeneration of myocardial fibers and areas of fibrosis were present both with and without associated vascular changes. One of the rats killed at 7 weeks showed a thrombus in a necrotic arteriole with an infarct of the surrounding myocardium. The vessel walls showing the changes of acute fibrinoid necrosis contained both PAS-positive material and sudanophilic lipid. The ascending thoracic aorta of 2 of the 5 rats killed at 14 weeks showed focal cartilaginous metaplasia of the wall with focal calcium deposition (Fig. 14).

Brain. No cerebral lesions were present in the control rats at either 7 or 14 weeks. The skulls of the rats with regenerated adrenal glands killed at 7 weeks were bulging and the suture lines widened. The brains of these rats were swollen and edematous, and 5 of the 6 showed gross cysts and focal hemorrhages. Microscopically, edema was manifest by spaces in a loose meshwork and the cysts were old infarcts with surrounding focal hemorrhage and extensive neuronal degeneration (Fig. 15). Smaller lesions consisted of focal hemorrhages and areas of neuronal degeneration without loss of cerebral substance. Acute fibrinoid necrosis and, in some cases, secondary thrombosis of arterioles supplying these areas accounted for the lesions (Fig. 16). The skulls of the rats killed at 14 weeks did not bulge and only minimal edema and focal neuronal degeneration were observed on microscopic study of the brain.

Pancreas and Mesentery. There were no lesions in the vessels of the pancreas and mesentery of the control rats at either 7 or 14 weeks. In the adrenal-enucleated rats, 5 of 6 killed at 7 weeks had lesions resembling periarteritis nodosa of pancreatic and/or mesenteric arteries (Fig. 17), while only 1 of 5 rats had such lesions after 14 weeks.

In general, the arterial changes differed from the arteriolar changes in the character and magnitude of the inflammatory reaction. Typical fibrinoid necrosis was present in small arteries and arterioles of the pancreas and mesentery just as in other parts of the body. The lesions of the arteries consisted of extensive "hyaline or fibrinoid" material immediately beneath the swollen endothelium, while the remainder of the arterial wall and adventitia were disrupted by an inflammatory reaction consisting of polymorphonuclear leukocytes, lymphocytes, mononuclear cells, eosinophils, and proliferating fibroblasts. Small PAS-positive globules were observed in the midst of the inflammatory reaction just outside the internal elastica (Fig. 18).

Pituitary Body. Unfortunately, the microscopic study of the an-

terior lobe of the pituitary body cannot be considered definitive in this experiment because of unsatisfactory fixation and consequent inadequate differentiation of cell types. In so far as could be determined, there were no significant changes in the pituitary glands of adrenal-enucleated rats, but it is necessary to conduct additional studies on this aspect of the problem in order to obtain satisfactory observations.

Other Organs. Arteriolar sclerosis and fibrinoid necrosis were present to a variable degree in the liver, spleen, thymus, and ovaries of adrenal-enucleated rats. Ovarian function seemingly was normal in the adrenal-enucleated rats inasmuch as there were numerous follicles in various stages of maturation and corpora lutea undergoing involution.

Discussion

The observation that hypertension and widespread vascular lesions develop following unilateral adrenalectomy and contralateral adrenal enucleation in uni-nephrectomized, salt-treated rats provides investigators with a new method for the produtcion of these changes in this species.

Furthermore, it provides some additional information relative to the participation of the adrenal cortex in the genesis of hypertension and vascular disease. According to Selye's hypothesis,[2,9] non-specific stress produces these diseases by way of an abnormal secretory response of the adrenal cortex. Therefore, one of the more interesting aspects of the present experiment is the demonstration that exogenous non-specific stress need not be an essential component of the pathogenetic chain leading to hypertension and vascular disease. Rather, the assumed abnormality of adrenal cortical function in this experiment is an accompaniment of regeneration of the cortex that follows operative enucleation, and, hence, the hypertension and vascular lesions are not the consequence of an external environmental stress. That non-specific stress was not a factor in this experiment was evidenced by the presence of normal follicles and corpora lutea in the ovaries of the adrenal-enucleated rats. Gonadal atrophy and functional quiescence normally result from exposure to non-specific stress and have been ascribed to a "shift" in hormone secretion of the anterior hypophysis in favor of adrenocorticotrophic hormone at the expense of gonadotrophic hormone output.[1] Thus, in the absence of evidence indicating such a "shift" in hormone secretion in the adrenal-enucleated rats, non-specific stress appears not to have been a factor.

It is apparent that most of the morphologic changes observed in this form of experimental hypertension are similar to those which may accompany hypertension in this species produced by desoxycorticosterone acetate,[2] lyophilized anterior pituitary substance (LAP) or anterior pituitary extract (APE),[3] cold,[4] cortisone,[9] somatotrophic hormone,[10] methylandrostenediol, [11] desoxycorticosterone acetate and renin,[12] cortisone and renin,[13] Reichstein's compound "S",[14] desoxycorticosterone acetate and angiotonin,[15] or following any of the numerous surgical procedures on the kidney such as cellophane[16] or collodion[17] wrapping or figure-of-eight ligature.[18] The lesions of the arterioles consisted of obliterative hyperplastic sclerosis and acute fibrinoid necrosis like that seen in malignant hypertension in the human being. Similarly, the kidneys showed acute necrosis of glomeruli with hemorrhage into the glomerular spaces and tubules; and the presence of hemosiderin in tubular cells indicated earlier episodes of hemorrhage. Unlike malignant hypertension in man, "adrenal-regeneration hypertension" of rats often is characterized by periarteritis nodosa-like changes of pancreatic and mesenteric arteries. The observation that occasional glomeruli in the control rats were undergoing necrosis indicates that rats at this age may be particularly susceptible to the damaging effects of excess sodium, as has been suggested by Meneely, Tucker, Darby, and Auerbach.[19]

Of particular interest in the present experiment were the cerebral lesions in the rats with regenerated adrenal glands and sacrificed after 7 weeks. These consisted essentially of edema, focal hemorrhage, and infarction as results of hyalinization, necrosis, and secondary thrombosis of cerebral arterioles, and were quite the same as those observed in rats treated with desoxycorticosterone acetate.[20,21] The cerebral lesions were most pronounced in those animals in which hypertension appeared earlier and was more severe. In each instance the estimated age of the lesions correlated well with the clinical onset of excitability, sensitivity to handling, clonic contractions of the extremities, and epileptoid convulsions, which occurred between the fourth and seventh weeks. The bulging of the calvarium, which was the result of the cerebral lesions, occurred because the suture lines had not yet closed in the young animals used in this experiment. This fact may account for the survival of these rats long after the onset of clinical signs of cerebral lesions, as suggested by Timiras, Faribault, and Selye.[21]

Another interesting observation was cartilaginous metaplasia of the

thoracic aorta in 2 of the 5 rats with regenerating adrenal glands and killed at 14 weeks. No such change has been reported previously in young, hypertensive rats, although it has been observed by Ingle and Baker[22] in female breeders 12 to 24 months old, from the Sprague-Dawley farms.

The origin of the regenerated adrenal cortex and the stimulus to regeneration are of interest. It is well known that following the procedure of adrenal enucleation as employed here, the cortex rapidly regenerates so that it approximates the original volume in about 1 month. Whether the regenerated cortical tissue is derived entirely from the residual cells of the zona glomerulosa or by transformation of capsular fibroblasts into glomerulosa cells and thence into the cells of the zona fasciculata and zona reticularis[23-25] is at present the subject of some debate. Nonetheless, it is acknowledged that, regardless of the origin of the cortical cells, the three zones of the adrenal cortex are reconstituted in the regenerated gland and that this is a direct result of stimulation by adrenocorticotrophic hormone from the anterior hypophysis.[26]

In the adrenal-enucleated rats killed at both the 7- and 14-week intervals, all three zones were present in the regenerated cortex. The zona glomerulosa was narrow, sometimes virtually unrecognizable, and consisted of small cells with dark nuclei and little cytoplasm. In this regard it differed little from the appearance of the zona glomerulosa in the regenerated adrenal glands of normal rats as reported by Greep and Deane[26] or from those of rats with diabetes insipidus as reported by Jones and Wright.[27] However, the zona glomerulosa of the regenerated adrenal glands contained an appreciable amount of lipid while in the controls this zone virtually was free of sudanophilic material. This sudanophilia of the glomerulosa in regenerated adrenal glands is reminiscent of that observed by Deane and Masson[28] in rats treated with renin or with renal hypertension, although the narrow width of the zone is in marked contrast to the thickened glomerulosa which they observed.

Although the mechanism by which the regenerating adrenal cortex is linked etiologically with the production of hypertension and cardiovascular-renal lesions is not yet clear, there are reasons for thinking that such a relationship is likely. That the regenerated cortex can secrete gluco-corticoids in normal or increased quantities has been demonstrated by Greep and Deane,[26] Brownell and Hartman,[29] and Ingle, Li, and Evans.[30] Furthermore, the facts that rats with regener-

ated adrenal glands do not require extra sodium chloride,[26] have relatively normal serum electrolyte values,[31] and prefer water to saline solution if allowed self-selection[32] have been considered presumptive evidence that these glands secrete mineralo-corticoids. In this experiment moderate atrophy of the thymus and retardation of growth in the adrenal-enucleated rats suggest excess secretion of gluco-corticoids since these steroids are known to produce such effects, but the simultaneous production of periarteritis nodosa suggests that mineralo-corticoids also are secreted in excess, since these lesions are known to be produced by desoxycorticosterone acetate and inhibited by gluco-corticoids such as cortisone. Perhaps the simultaneous development of these divergent effects may be explained by the overproduction of a single steroid such as aldosterone, which has been shown to possess both gluco-corticoid and mineralo-corticoid activity. However, it is also possible that the hypertension and vascular lesions may result from some other substance, possibly of pituitary or renal origin, acting on tissues which have in turn been sensitized or conditioned by the secretions of the regenerating adrenal cortex.

Lastly, the regenerated adrenal cortices frequently showed intracytoplasmic PAS-positive globules. This peculiar cellular change has been observed in the adrenal cortex of rats treated with lyophilized anterior pituitary substance alone and in combination with methyltestosterone, desoxycorticosterone acetate, and thyroxine,[33] methylandrostenediol,[11] and ACTH.[34] The globules have been interpreted to be the result of storage of secretory substances,[33] increased cellular functional activity,[34] or cellular degeneration.[11] Since, in this experiment, they were associated with areas of cortical necrosis and PAS-positive material subjacent to capsular arterioles with fibrinoid necrosis, they could result from either degeneration of the cells or phagocytosis of material derived from the damaged wall of the degenerating arteriole. The presence of globules having similar tinctorial characteristics in the wall of mesenteric arteries with periarteritis nodosa and renal arteries undergoing fibrinoid necrosis suggests that the intracytoplasmic globules in the adrenal cortex are of vascular origin.

Summary

Unilaterally nephrectomized rats allowed 1 per cent sodium chloride to drink developed hypertension and widespread vascular disease following unilateral adrenalectomy and contralateral adrenal enucleation. The arteriolar lesions in the kidney, heart, brain, adrenal capsule,

mesentery, pancreas, thymus, and ovary resembled the fibrinoid arteriolar necrosis of malignant hypertension in the human being. The renal changes also included glomerulonecrosis, proliferation of glomerular epithelial cells, hemorrhage into the nephron, thickening of the tubular basement membrane, dilatation of tubules, and hyaline casts. The cerebral lesions consisted of infarcts and focal neuronal degeneration, hemorrhages, and edema with bulging of the calvarium. Arteries in the mesentery and pancreas showed lesions which have been called "periarteritis or panarteritis nodosa." Cartilaginous metaplasia was observed in the thoracic aorta of 2 hypertensive rats killed after 14 weeks.

The regenerated adrenal glands showed all three zones, but the zona glomerulosa was thin and was composed of small cells containing lipid in contrast to the thicker zona glomerulosa of control rats which was virtually free of sudanophilic lipid. Focal cortical necrosis was present in regenerated adrenal glands associated with fibrinoid degeneration of capsular arterioles. Intracytoplasmic PAS-positive globules were present in cortical cells adjacent to areas of necrosis.

The experiment provides a new method for the production of experimental hypertension and vascular disease in the rat. It strongly supports the hypothesis that altered adrenocortical function is of fundamental importance in the pathogenesis of this disease. In addition, it demonstrates that non-specific, exogenous stress is not needed to produce experimental disease of this type, and indicates that the stimulus to abnormal adrenocortical function can lie entirely within the organism.

I wish to thank Miss Betty Jean Wilson for the preparation of the histologic slides and Mr. Carl Bishop, Department of Pathology, Duke University, for the photomicrographs.

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LEGENDS FOR FIGURES

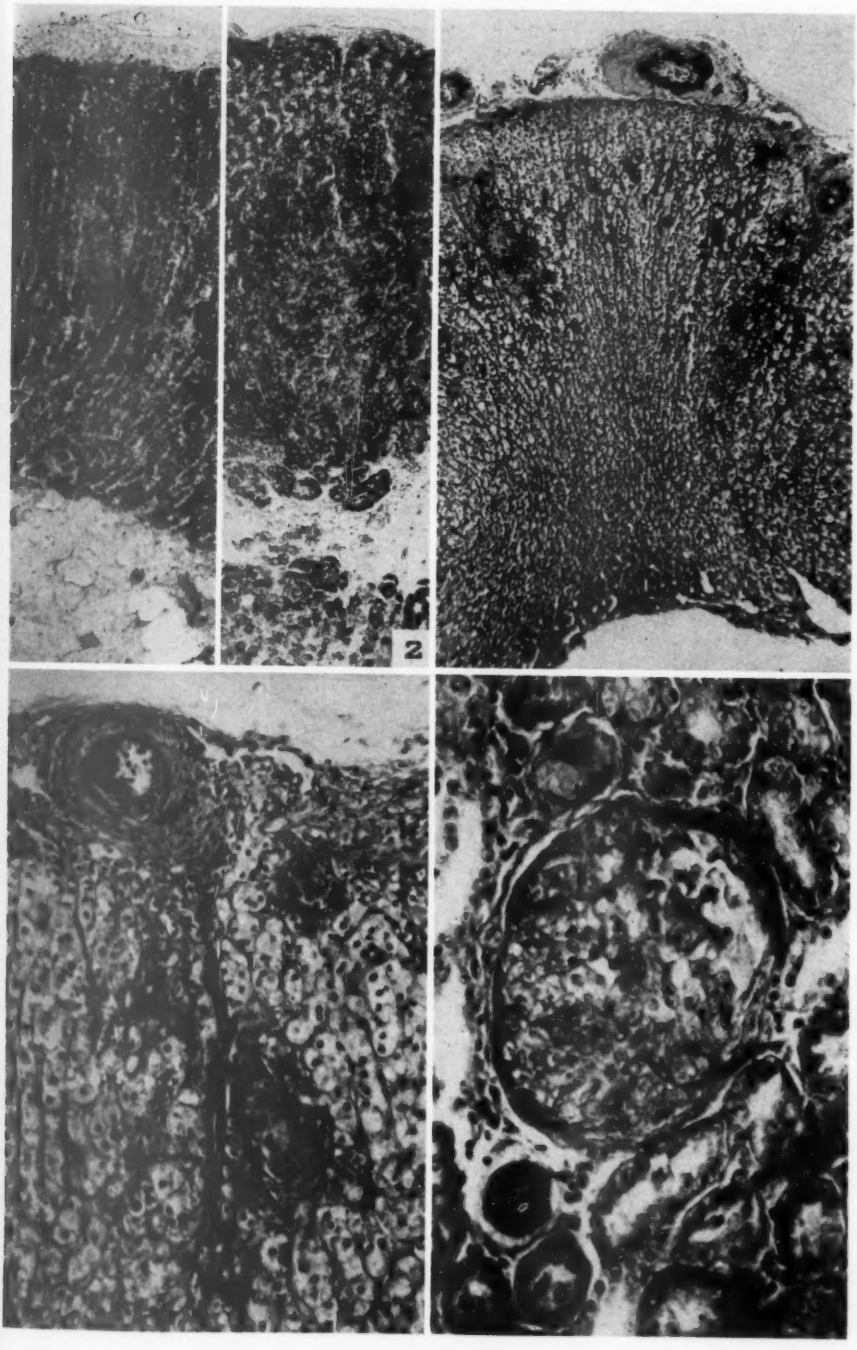
FIG. 1. Adrenal cortex of control rat, 7 weeks. Normal sudanophilia of zona reticularis and zona fasciculata, and virtual absence of lipid in zona glomerulosa. Sudan IV stain. × 82.

FIG. 2. Adrenal cortex of adrenal-enucleated rat, 7 weeks. Sudanophilia of zona reticularis and zona fasciculata is slightly less than in adrenal cortex of control rat, but zona glomerulosa is laden with fat. Sudan IV stain. × 82.

FIG. 3. From the same adrenal gland as that from which Figure 2 was taken, showing fibrinoid necrosis of capsular arterioles and focal necrosis of cortex. Thin zona glomerulosa and clear cytoplasm of cells in the zona reticularis and zona fasciculata. McGregor's stain. × 82.

FIG. 4. Focal necrosis in regenerated adrenal cortex, 7 weeks, with PAS-positive globules in the cytoplasm of adjacent cortical cells. Periodic-acid Schiff's (PAS) stain. × 188.

FIG. 5. Most severely damaged glomerulus found in any of the control rats. Slight necrosis of capillaries and protein in Bowman's space. McGregor's stain. × 331.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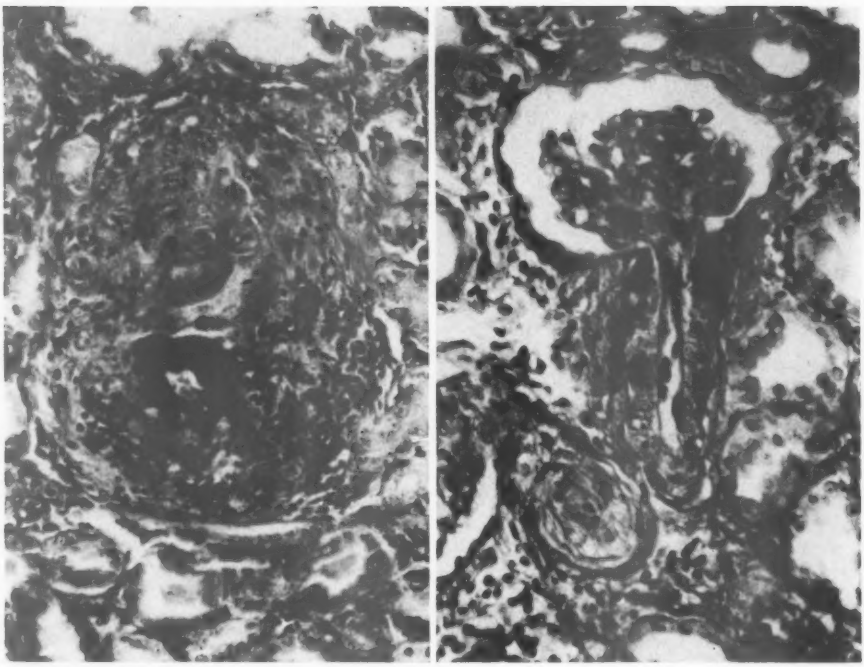


Fig. 6. Typical damaged glomerulus of adrenal-enucleated rat, 14 weeks. Fibrinoid necrosis of capillary tufts and hemorrhage. McGregor's stain. × 297.

Fig. 7. Glomerulus with afferent arteriole in adrenal-enucleated rat, 7 weeks, showing fibrinoid necrosis. McGregor's stain. × 348.

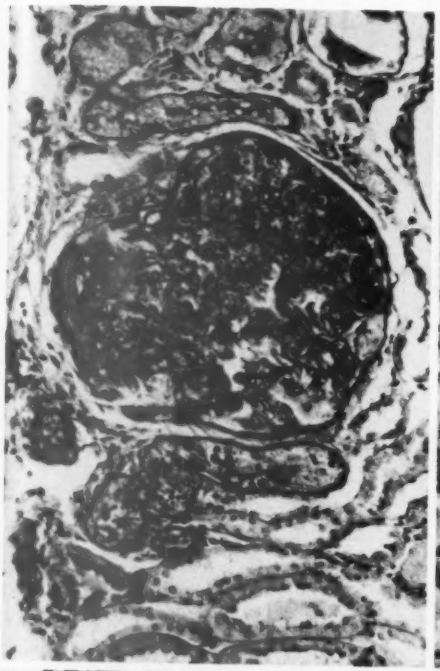
Fig. 8. Glomerulonecrosis in kidney of adrenal-enucleated rat, 7 weeks, with droplets of protein in adjacent convoluted tubule. PAS stain. × 305.

Fig. 9. Hemosiderin in tubules of kidney of adrenal-enucleated rat, 7 weeks. Prussian blue reaction. × 196.

Fig. 10. Fat in glomerulus and convoluted tubules of kidney of adrenal-enucleated rat, 7 weeks. Sudan IV stain. × 162.

Fig. 11. Fat in wall of renal arteriole with fibrinoid necrosis in adrenal-enucleated rat, 7 weeks. Sudan IV stain. × 348.

Fig. 12. Fibrinoid necrosis of arterioles in atrial wall of heart of adrenal-enucleated rat, 7 weeks. McGregor's stain. × 166.



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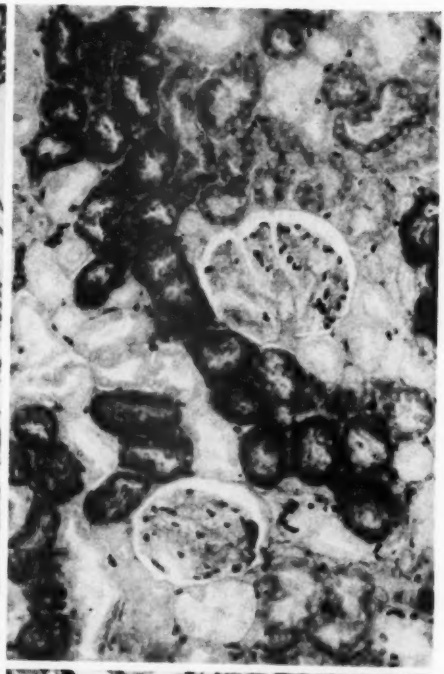

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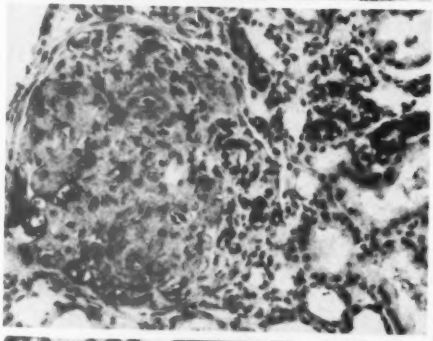

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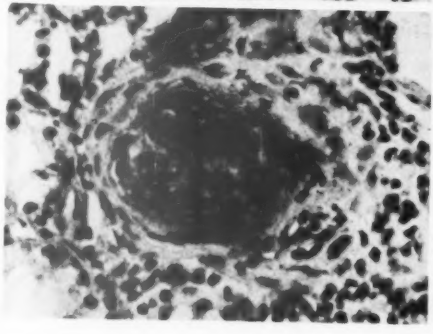

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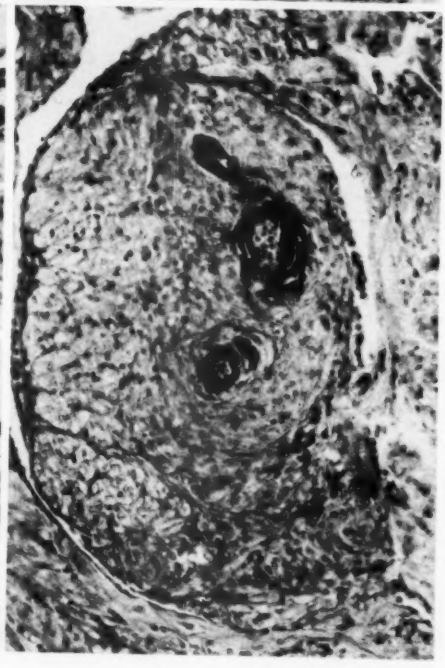

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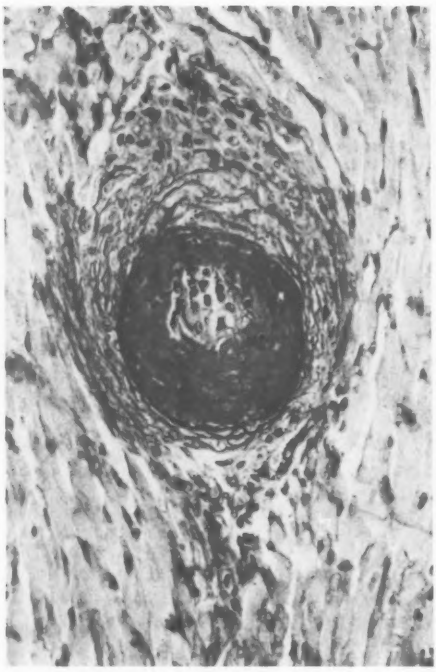

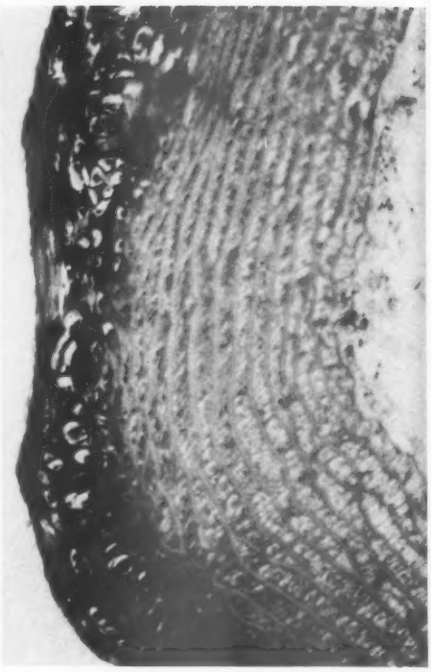

FIG. 13. Adrenal-enucleated rat, 7 weeks. Fibrinoid necrosis and thick layer of subendothelial fibrinoid material in arteriole of ventricular wall of heart. PAS stain. × 322.

FIG. 14. Cartilaginous metaplasia of aorta of adrenal-enucleated rat, 14 weeks. PAS stain. × 175.

FIG. 15. Edema, loss of neurons, and cyst formation in brain of adrenal-enucleated rat, 7 weeks. PAS stain. × 35.

FIG. 16. Fibrinoid necrosis and thrombosis of cerebral arterioles in adrenal-enucleated rat, 7 weeks. Phosphotungstic acid hematoxylin stain. × 408.

FIG. 17. Periarteritis nodosa of mesenteric artery of adrenal-enucleated rat, 7 weeks. PAS stain. × 57.

FIG. 18. Mesenteric artery of adrenal-enucleated rat, 7 weeks. Periarteritis nodosa showing PAS-positive globules in cells of the media. PAS stain. × 764.

FIG. 19. Renal arteriole showing PAS-positive globule in media. Adrenal-enucleated rat, 7 weeks. PAS stain. × 743.

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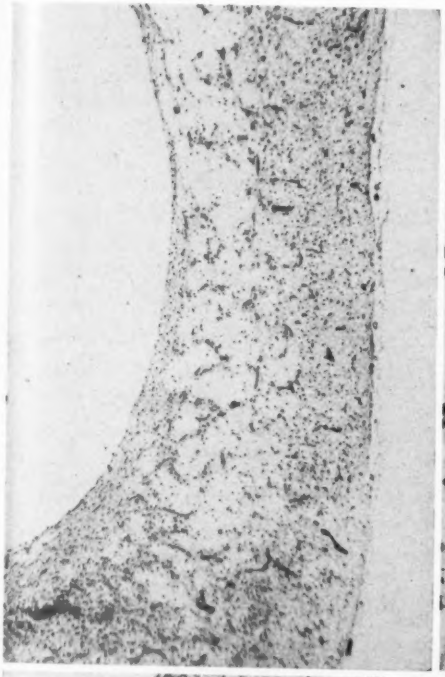

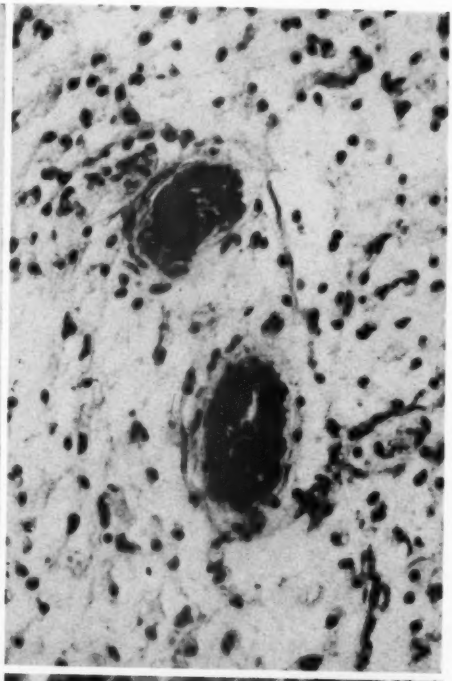

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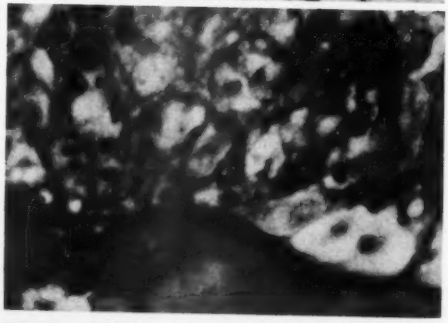

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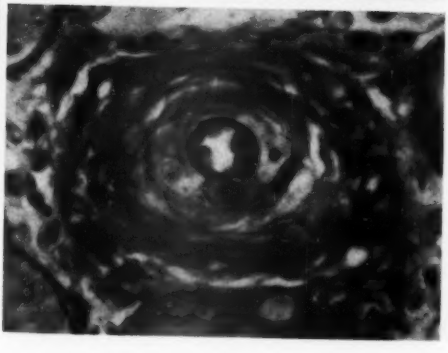

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THE ENDOCRINE SIGNIFICANCE OF HYPOPHYSEAL TUMORS IN MAN*

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Hypophyseal tumors of two common types have been accepted in man: "acidophil adenomas" which are associated with somatic overgrowth and manifestations of endocrine hyperactivity, and "chromophobe adenomas" which, although without endocrine function themselves, may lead to "hypopituitarism," allegedly through compression atrophy of other portions of the hypophysis.[1] However, patients are encountered occasionally who cannot be fitted readily into these categories.

Twenty-seven patients with hypophyseal disease were studied in order to determine, first, to what extent cytologic examination of the hypophysis justifies the clinical distinction between "acidophil" and "chromophobe" tumors, and, second, to review the anamnestic and anatomical data with respect to other endocrine organs.

MATERIAL AND METHODS

Pathologic material available at the Massachusetts General and Beth Israel Hospitals, Boston, Massachusetts, comprised 8 patients with somatic overgrowth (one man and 6 women with acromegaly as well as a non-acromegalic woman 185 cm. tall) and 19 with large "chromophobe adenomas" (12 men and 7 women). Pertinent clinical and anatomical data are summarized in Tables I and II.

Sections of hypophysis were stained with hematoxylin and eosin, modified Mallory's aniline blue, and the periodic acid-Schiff technique with orange G counterstain. Cells were classified according to cytoplasmic granulation and nuclear characteristics by the method previously described.[2] Typical cells may be characterized as follows:

Basophils. Granules numerous and intensely Schiff positive(Fig. 1).

Acidophils. Granules numerous and Schiff negative but staining with fuchsin or orange G (Fig. 2).

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TABLE I

Clinical and Anatomical Findings in Patients with Somatic Overgrowth

No.	Age	Sex	Duration	Onset	Therapy	Hypophysis	Adrenal glands	Gonads	Thyroid gland	Pancreas	Parathyroid glands	Uterus	Breast	Associated conditions	Cause of death
			yrs.												
1 MGH 12,704	50	M	23	Decreased libido	X-ray, 12 and 5 yrs.; estrogens, 4 yrs.; testosterone, 3 yrs.; none, 11 mos.	1.5 gm.; adenoma of multinucleate HA; Amp, few Ac	24 gm.; nodular hyperplasia; 17-ks, 5.3 to 12.3 mg.	Total atrophy; FSH, +7 m.u., negative after estrogen	41 gm.; fetal adenomas, colloid goiter	150 gm.; abundant large islands; FBS, normal; GTT, equivocal	10 x 5 mm.; abundant chief cells, clumps of oxyphils			Bleeding duodenal ulcer, hypertension	Cerebrovascular accident
2 MGH 13,468	25	F	8	Amenorrhea, hot flashes	Insulin, 6 yrs.; transsphenoidal resection, 3 yrs.; estrogens, 6 yrs. to death	2 x 1.5 x 1.8 cm.; adenoma; small Ac, rare B, pyknotic nuclei	18 gm.; moderate hyperplasia; 17-ks, 7.7 to 21.3 mg.	Active stroma, follicle cysts, no corpora lutea; FSH, +7 m.u., negative after estrogen	19 gm.; focal activity; BMR, −8 and +15	80 gm.; abundant islands; FBS, normal; GTT, diabetic	Acinar hyperplasia of chief cells	No data	Active lactation	Acne, pigmented spots on skin, hirsutism	Meningitis
3 BIH S49-922	32	F	7	Irregular menses	Stilbestrol, none for 1 yr.; transsphenoidal resection	Large tumor invading sphenoid; HA, Amp	No data	Scanty menses for 7 yrs., improved by stilbestrol	No data	No data	No data	No data	No data	Central obesity	Patient living
4 BIH A47-70	47	F	1	Amenorrhea	Insulin	Large tumor destroying sella and compressing brain; Amp, rare HA	"Normal size"; nodular hyperplasia	Inactive stroma, corpora albicantia	"Normal size"; cuboidal epith., fetal adenomas, exophthalmos	140 gm.; many large islands, minimal fibrosis	4 x 1 x 2 mm.; normal histology	Fibroids	Epithelial proliferation, microscopic secretion	Diabetes, obesity, hirsutism, cutaneous fibromas	Diabetic coma
5	58	F	25	Irregular	Craniotomy,	3 cm. diam-	Nodular	Atrophic	28 gm.;	50 gm.;	Chief cells,				

5 MGH 10,473	58	F	25	Irregular menses	Craniotomy, 25 yrs.; x-ray, 9, 4, and 3 yrs.; radium, 1 yr.; thyroid, 3 yrs. to death	3 cm. diameter; adenoma invading brain; Chr, few Amp, HA, Ac	Nodular hyperplasia	Atrophic ovaries, corpora albicantia, FSH, negative	28 gm.; diffuse fibrosis, low epithelium	50 gm.; islands normal; FBS, normal; GTT, diabetic	Chief cell hyperplasia, many oxyphils	Cystic hyperplasia of endometrium, polyps	Dilated ducts, lobular hyperplasia	Hemangioma, cholangioma, myxedema	Tracheobronchitis
6 MGH 16,731	67	F	c.20	Menopause, hot flashes	Insulin, 11 yrs.; KI, 7 yrs.; I-131, 7 yrs.; x-ray, 6 yrs.; no therapy, 6 yrs.	0.60 gm.; no tumor, 5368 cells counted: Amp, 13.4%; HA, 2.8%; B, 18.3%; Ac, 16.8%; Chr, 48.4%; HyB, 0.3%	16 gm.; nodular hyperplasia, anisonucleosis	Moderate stromal hyperplasia	"Large" nodular goiter, fibrosis and calcification; exophthalmos; BMR, +72 and +94	75 gm.; islands normal	Acinar hyperplasia of chief cells, many oxyphils	Atrophic endometrium	No data	Diabetes, hirsutism, thyrotoxicosis, hypertension, arthritis, lipoma, osteoma	Cerebrovascular accident
7 MGH 10,057	74	F	c.30	Oophorectomy	None	2.25 gm.; 8 mm. adenoma; Amp, HA, few Ac	Nodular hyperplasia	Surgically absent, 30 yrs.	Not examined	100 gm.; islands normal	Not examined	Active glands in cervical stump	No data	Ca. of colon, meningioma, neuroma, lipoma	Cerebrovascular accident
8 MGH 13,929	40	F	?	Post-mortem diagnosis	900 mg. testosterone 3 wks. before death	1.3 x 0.6 cm.; 0.6 x 0.4 cm. adenoma of Amp, HA, Ac, Chr	27 gm.; moderate nodular hyperplasia	Lt., simple cysts; Rt., normal stroma and degenerating corpus luteum	16 gm.; focal involution	60 gm.; adenomatous hyperplasia of islands	Not examined	Normal proliferative endometrium	Sclerosing adenosis	185 cm. tall, hirsutism, renal calculi, mucous colitis, ileal polyp, acute myelogenous leukemia	Acute myelogenous leukemia

Amp = Sparsely granulated amphophil
HA = Hypertrophic amphophil
Ac = Acidophil
B = Basophil
Chr = Chromophobe
HyB = Crooke's hyaline basophil
FBS = Fasting blood sugar
GTT = Glucose tolerance test
BMR = Basal metabolic rate

FSH = Follicle stimulating hormone in mouse units (m.u.); normally 6-12 m.u. for adults of reproductive age and 50 m.u. or more for post-menopausal women
17-ks = 17-Ketosteroids in mg. per 24 hours; normally 4-8 mg. for adult women and 12-20 mg. for adult men
PBI = Protein-bound iodine

Amphophils. Granules sparse, weakly Schiff positive, and staining variably with Mallory's technique (Fig. 1).

Hypertrophic Amphophils. Agranular cells with giant nuclei (Fig. 1).

Chromophobes. Agranular cells with small nuclei (Figs. 1 and 2).

The material was not suitable for the determination of cellular composition of the hypophyses outside of the tumors. Sections of the endocrine glands other than the hypophysis were stained with hematoxylin and eosin.

Hypophysis

Somatic Overgrowth (Patients 1 to 8, Table I). Seven patients with somatic overgrowth had tumors of the hypophysis. Four tumors were discrete and intrasellar; three were extrasellar, either compressing or frankly invading surrounding structures. In five of these seven tumors (cases 1, 3, 4, 7, 8), the sparsely granulated and hypertrophic amphophils rather than the acidophils constituted the dominant cell type (Figs. 3 to 6). It is suggested, therefore, that the amphophils rather than the acidophils are the source of growth hormone.

In two tumors there was a different picture in that only a few amphophils were present. Patient 5, having received thyroid medication for 3 years until death, had an extrasellar invasive tumor composed predominantly of agranular chromophobes with small nuclei. Patient 2 had a discrete intrasellar adenoma composed largely of tiny, well granulated acidophils with pyknotic nuclei (Figs. 7 and 8). In fact, this last patient was the only one of the series showing the traditional acidophilic adenoma of somatic overgrowth. However, the patient had been on stilbestrol prior to death, a medication which resulted in a distinct suppression of growth hormone (reduction in blood phosphorus, in growth of axillary hair, and in volume of hands and feet). We consider it possible that the cellular composition of these two hypophyseal tumors resulted from the hormonal medication; this is in keeping with previous observations indicating a suppressive effect of thyroid[3] and stilbestrol[4,5] upon the amphophils in non-tumorous hypophyses.

The hypophyseal tumors which had been irradiated showed cellular atypicality and nuclear pleomorphism, but the cell types remained identifiable and were similar to those seen in the non-irradiated tumors (Fig. 6).

The eighth patient (case 6), a classical acromegalic, showed neither tumor nor enlargement of the hypophysis. There was, however, a threefold increase of the amphophils, a two-fold increase of the basophils, and a reduction of the acidophils to half the expected value (Figs. 1, 2, and 9). In a case of Klinefelter's syndrome that we have previously

reported,[6] diffuse hyperplasia of the amphophils was observed in association with mild acromegaly, but the proportion of acidophils was within normal limits. Acromegaly in patients with non-tumorous hypophyses of normal size has been reported also by others.[7] All these observations indicate that non-tumorous hyperplasia of amphophils may be as productive of excessive growth hormone as hypophyseal tumors.

"Chromophobe Adenomas" (Patients 9 to 27, Table II). The dominant cell type in most of the 19 "chromophobe adenomas" was again the sparsely granulated amphophil (Figs. 10 and 11). In some, but by no means all, of these patients the tumor cells were smaller and the nuclei more uniform than those characteristically seen in acromegaly (cf. Figs. 3, 5, and 10). Following administration of testosterone, thyroid, ACTH, cortisone, or crude adrenal extract, the proportion of agranular chromophobes with small pyknotic nuclei was increased (Fig. 12).

Four of the 12 men with the clinical diagnosis of "chromophobe adenoma" had physical or radiologic evidence of mild somatic overgrowth (cases 9, 10, 15, and 16).

Adrenal Glands

Somatic Overgrowth. The adrenal glands were large, with a combined weight of from 16 to 27 gm., and all showed nodular cortical hyperplasia.[8]

In one woman the 17-ketosteroid excretion was elevated and became reduced following the administration of estrogen (case 2). The excretion was low in the patient who had received prolonged thyroid medication (case 5). It was within normal limits for the single male of this series (case 1).

Hirsutism was recorded for 4 women.

"Chromophobe Adenomas." Although patients with "chromophobe adenomas" frequently are considered to have "panhypopituitarism," the adrenal glands, like the hypophyses, resembled the glands of the acromegalic patients. Adrenal weight was increased in the majority, ranging from 13.5 to 30 gm. Nodular cortical hyperplasia was present in 11 patients. One woman (case 24) had a well defined cortical adenoma, 1 cm. in diameter. There were only 3 cases in which adrenal weight was below normal (cases 10, 12, and 27).

Three patients (cases 21, 22, and 25) had, variously, central obesity, hypertension, diabetes mellitus, and hirsutism, i.e., elements of Cushing's syndrome. In one patient (case 21) the 17-ketosteroid excretion

TABLE II

Clinical and Anatomical Findings in Patients with "Chromophobe Adenomas"

No.	Age	Sex	Duration	Onset	Therapy	Hypophysis	Adrenal glands	Gonads	Thyroid gland	Pancreas	Parathyroid glands	Prostate or Uterus	Breast	Associated conditions	Cause of death
9 BIH A41-37	41	M	yrs. 3	Impaired vision	Craniotomy, 3 days	4.5 x 4.5 cm.; tumor eroding sphenoid and extending to base of brain; Amp, HA	"Normal size"; nodular hyperplasia	Hypospermatogenesis, tubular sclerosis, abundant Leydig cells	"Large"; focal involution and hyperplasia	"Normal size"; abundant islands, some very large	No data	High columnar epithelium with papillary infolding		Fibromas of skin; polyposis of colon; large bones; large hands and feet; jaw normal	Craniotomy
10 MGH 15,148	48	M	13	Decreased libido, hot flashes	X-ray, 11 yrs. and terminally; thyroid and testosterone, 11 yrs.; ACTH, cortisone, testosterone, terminally	17 gm.; huge, hemorrhagic Amp tumor with pyknotic nuclei	8.7 gm ; cortex normal; 17-ks, 0.8 mg. terminally	21 gm.; severe hypospermatogenesis; no Leydig cells; FSH, negative terminally	14.5 gm.; low epithelium, pyknotic nuclei; BMR, −20 and −33	100 gm.; abundant, large islands; FBS, normal	Normal	Chronic prostatitis, low pyknotic epithelium		Myotonia atrophica; osteoma of skull; large hands and feet; spacing of teeth; decreased body hair	Spontaneous hemorrhage into hypophysis
11 MGH 15,473	51	M	9	Headaches	Craniotomy, terminally; 125 mg. cortisone, terminally	Large tumor of Amp, HA, with few Ac peripherally	14 gm.; slight nodular hyperplasia	Rt., 14 gm.: total atrophy (mumps orchitis); Lt., 35 gm.: active spermatogenesis and Leydig cells; FSH, 13 m.u.	20 gm.; high epithelium, mild fibrosis, lymphocytic infiltration; BMR, +3 and +8	110 gm.; abundant islands, some large; FBS, normal	Dense chief cells, little fat	High columnar epithelium, papillary infolding	Gynecomastia with microscopic secretion	Rheumatoid arthritis	Craniotomy
12 MGH 12,893	52	M	3	Myxedema	Craniotomy, 3 days; lipoadrenal extract, ACTH, DOCA, 3 days	5 x 4 x 3.5 cm.; Amp with small, pyknotic nuclei; necrotic areas	9 gm.; thin cortex; 17-ks, 2.0 mg.	Few mature sperm and Leydig cells, some tubular fibrosis; FSH, +13 m.u.	9 gm.; diffuse fibrosis, lymphocytes, cuboidal to high epithelium; BMR, −29; PBI, 2.6 gamma %	75 gm.; normal islands; FBS, normal; GTT, flat	Normal	Benign prostatic hypertrophy		Decreased body hair	Craniotomy
13 BIH A52-37	53	M	12	Impaired	Crani-	2 x 1 x 0.6	No data	No data	No data	No data	No data	No data			Patient

									PBI, 2.6 gamma %							
13 BIH S51-2406	53	M	12	Impaired vision	Cranio-otomies, 12 yrs., 5 yrs.; trans-sphenoidal resection, present admission	2 x 1 x 0.6 cm. tumor removed; Amp with small nuclei	No data	No data	No data	No data	No data	No data			Patient living
14 MGH 12,781	54	M	9	Impaired vision	X-ray, 5 yrs.; cranio-tomy, terminally	Large tumor, Amp, Chr, rare HA	24 gm.; nodular hyper-plasia	Mature sperm, Leydig cells, some tubular thicken-ing	"Normal size"; active acini	120 gm.; many large islands	No data	Acute prostatitis		Diabetes	Craniotomy
15 BIH S46-959 S51-3285	55	M	15	Headache	X-ray, 5 yrs.; trans-sphenoidal resection, 5 yrs. and present admission	Large tumor eroding sella; Amp, rare HA	17-ks, 7 mg. on 1st adm.; 2.2 mg. on 2nd adm.	Decreased libido	BMR, +9	GTT, normal	No data	No data		Obesity; osteo-porosis; large hands and jaw; decreased body hair; fibroma, skin	Patient living
16 MGH 9,238	55	M	10	Decreased libido	Cranio-otomies, 7 yrs. and termin-ally; x-ray, 1 mo.; adrenal extract, thyroid, pituitrin, antuitrin S, terminally	3.5 x 2.5 cm.; Amp-Chr tumor with rare Ac; mitotic figures	15 gm.; recent hemor-rhagic infarction	Atrophy, fibrosis	9 gm.; severe fibrosis, focal lym-phocytes, cuboidal epith-elium; BMR, −26	Normal islands; FBS, 180 mg. %; GTT, equivocal	Large but with much fat	Normal size; inactive epith-elium		Skeletal x-rays suggest acro-megaly; decreased body hair; diabetes	Craniotomy
17 BIH S49-3039	60	M	1	Impaired vision	Trans-sphenoidal resection	Sella, 2.3 cm.; tumor invading sphenoid; Amp with small, uniform nuclei	Eosinophil count, 312; 206 after epi-nephrine	Decreased libido	BMR, −38 and −34	No data	No data	No data		Kyphosis and scoliosis; obesity; decreased body hair	Patient living

TABLE II (continued)

No.	Age	Sex	Duration	Onset	Therapy	Hypophysis	Adrenal glands	Gonads	Thyroid gland	Pancreas	Parathyroid glands	Prostate or Uterus	Breast	Associated conditions	Cause of death
18 MGH 15,117	62	M	*yrs.* ?	Post-mortem diagnosis	Insulin, 10 yrs.	1.5 x 2.0 cm. tumor eroding posterior clinoids; Amp, few Ac	20 gm.; nodular hyperplasia	Hypospermatogenesis, focal tubular atrophy, abundant Leydig cells	37 gm.; active epithelium, fetal adenomas, lymphocytic infiltration	130 gm.; normal islands	No data	Grossly normal		Diabetes; ca. and polyposis of colon; papillomas of skin; rheumatoid arthritis	Ca. of colon
19 MGH 9,945	72	M	40	Mumps orchitis	Testosterone, 2½ mos.	3 x 2.5 x 2 cm.; 1.5 cm. Amp adenoma with pyknotic nuclei, few HA	Narrow cortex; 17-ks, 1.2 mg.	Total atrophy, no Leydig or Sertoli cells; FSH, negative	10 gm.; fibrosis, low epithelium; BMR, −8 and −15	Grossly normal; FBS, 92 mg. %	No data	No data		Decreased body hair	Coronary thrombosis
20 MGH 15,990	77	M	?	Post-mortem diagnosis	None	2 gm.; Amp with rare Ac, HA	15 gm.; moderate nodular hyperplasia, focal anisonucleosis	Hypospermatogenesis, tubular thickening, few Leydig cells	24.5 gm.; hyperinvolution	150 gm.; large islands suggest adenomatous hyperplasia	"Large"; small papillary adenoma, large clumps of oxyphils	Grossly normal		Duodenal ulcer	Ruptured abdominal aneurysm
21 MGH 12,661	36	F	10	Menstrual irregularities	Craniotomy, terminally	1.5 x 1 x 1 cm. adenoma; HA, Amp, Chr	"Normal size"; severe nodular hyperplasia, anisonucleosis	"Small"; active stroma, thecomatosis; corpora albicantia, no ova or follicles	Not examined	140 gm.; islands normal; FBS, normal	No data	Tuberculous salpingitis	Simple mastectomy; mastopathia cystica, 10 yrs.	"Cushing's syndrome"; central obesity; hirsutism; hypertension	Craniotomy
22 MGH 11,535	41	F	12	Amenorrhea	Insulin, 4 yrs.; craniotomy, terminally	Amp adenoma	30 gm.; moderate nodular hyperplasia; 17-ks, 6.7 mg.	Active stroma, rare ova; corpora albicantia; FSH, >13 m.u., <48 m.u.	Focal involution, moderate fibrosis; BMR, −12 and −13	Normal	No data	Grossly normal	Persistent lactation, 10 yrs.	Central obesity; diabetes	Craniotomy
23 BIH S52-1045	48	F	1	Amenorrhea	X-ray and cortisone, 3 wks., followed by transsphenoidal resection	2 gm. tumor; pyknotic chromophobes	No data	No data	No data	No data	No data	No data	Milky discharge bilaterally	Hodgkin's (?) disease, "cured" by spray radiation, 20 yrs.	Patient living
24 MGH	69	F	?	Post-mortem	None	3 x 2 cm.; Amp	16 gm.;	Severe	17 gm.;	43 gm.;	Numerous	Cystic		Carcinoma;	Hyperten-	Arterio-

					sphenoidal resection										
24 MGH 14,075	69	F	?	Post-mortem diagnosis	None	3 x 2 cm.; Amp, HA, Chr with peripheral rim of small Ac	16 gm.; 1 cm. adenoma, nodular hyperplasia	Severe stromal hyperplasia, thecomatosis	17 gm.; active epithelium	43 gm.; numerous large islands	Numerous small clusters of oxyphils	Cystic hyperplasia, endometrium; cervical and endometrial polyps	Carcinoma; mastopathia cystica	Hypertension; ca. of breast; polyposis and leiomyoma of stomach	Arteriosclerotic heart disease; pulmonary emboli
25 BIH A53-145	77	F	?	Post-mortem diagnosis; oophorectomy, 35 yrs.	None	"Unusually large"; small tumor of relatively well granulated Amp	17.5 gm.; thick cortex, slight nodular hyperplasia	Surgically absent, 35 yrs.	21 gm.; involutional nodule	70 gm.; very large, abundant islands; acute pancreatitis	2 cm. chief cell adenoma, huge clumps of oxyphils; Ca, 11.3 mg. %; P, 2.9 mg. %	Surgically absent, 35 yrs.	Abundant lobules; secretion and intraductal hyperplasia	Central obesity; hypertension; decreased body hair; arthritis; cataracts; renal calculi	Pyelonephritis; uremia
26 MGH 15,702	80	F	?	Post-mortem diagnosis; total ovarian atrophy	None	2.3 gm.; tumor of Amp mixed with Chr, rare Ac, B	13.5 gm.; nodular hyperplasia, focal anisonucleosis	Totally atrophic, not found grossly	12 gm.; cuboidal high epithelium	60 gm.; abundant, large islands	No data	Hyperplastic endometrium; mitotic figures	Rt., carcinoma; Lt., intraductal hyperplasia, secretion	Hypertension; cataracts; duodenal ulcer; bronchial asthma; osteoarthritis; rheumatic heart disease; ca. of breast and bladder	Coronary thrombosis
27 BIH A53-139	87	F	?	Post-mortem diagnosis; amenorrhea for 53 yrs.	None	"Large"; 7mm. Amp adenoma; Amp hyperplasia in rest of gland	9 gm.; peripheral nodules, nodular hyperplasia, anisonucleosis	Severe atrophy	25 gm.; normal	7 cm. duct cell cystadenoma; abundant, very large islands suggest hyperplasia	Large oxyphil adenoma	"Infantile"	No data	Bladder calculi; arthritis; cystadenoma of pancreas	Arteriosclerotic heart disease; congestive failure

Amp = Sparsely granulated amphophil
HA = Hypertrophic amphophil
Ac = Acidophil
B = Basophil
Chr = Chromophobe
FBS = Fasting blood sugar
GTT = Glucose tolerance test
BMR = Basal metabolic rate

FSH = Follicle stimulating hormone in mouse units (m.u.); normally 6-12 m.u. for adults of reproductive age and 50 m.u. or more for post-menopausal women
17-ks = 17-Ketosteroids in mg. per 24 hours; normally 4-8 mg. for adult women and 12-20 mg. for adult men
PBI = Protein bound iodine

was elevated. It was normal in one (case 22) and depressed in 4 (cases 10, 12, 15, and 19).

There was decreased body hair in 6 males and one female.

Gonads

Somatic Overgrowth. The histologic features of the ovaries, known in 5 patients, were those of stromal hyperplasia twice and atrophy twice. The unduly tall non-acromegalic woman (case 8) had one ovary which was multicystic. The other ovary was seemingly functional in that it contained a degenerating corpus luteum. Menstrual irregularities were followed by amenorrhea in 3 of the 4 women who were less than 35 years of age at the onset of their disease. The fourth, the woman 185 cm. tall, had regular menses until the time of her death at the age of 40. In the remaining 3 women of the series, the disease became manifest subsequent to either a surgical or spontaneous menopause.

The male acromegalic patient (case 1) complained of decreased libido throughout his illness. At necropsy the testicular tubules were fibrosed, containing rare pyknotic Sertoli cells and no germinal epithelium. Leydig cells were absent.

Hot flashes were noted early in 2 patients (cases 2 and 6). Gonadotropin excretion was low in 2 patients when determined late in the course of the disease and disappeared entirely following estrogen therapy (cases 1 and 2).

"Chromophobe Adenomas." The ovaries were studied in 5 women with "chromophobe adenomas." There was hyperplasia of the stroma in 3 (cases 21, 22, and 24) and severe atrophy in 2 (cases 26 and 27).

Gonadal dysfunction often preceded the local manifestations of hypophyseal enlargement. In 3 of the 7 women of this group the menses ceased prematurely at ages 26 to 34 (cases 21, 22, and 27). It seems unlikely that amenorrhea was due to primary hypophyseal failure, because one of these patients (case 22) excreted normal amounts of gonadotropin after her illness had become well established. As in the somatic overgrowth group, symptoms of hypophyseal tumor appeared shortly after a spontaneous menopause in one patient (case 23) and after surgical castration in another (case 25).

Six of 9 patients in whom the testes could be studied showed bilateral hypospermatogenesis or aspermatogenesis (cases 9, 10, 16, 18, 19, and 20). In another man aspermatogenesis was confined to one testis and probably was related to previous mumps orchitis (case 11). In 2 patients mature spermatozoa were found in both testes (cases 12 and

14); Leydig cells were present in 6 men (cases 9, 11, 12, 14, 18, and 20).

Among the 12 male patients, gonadal deficiency clearly preceded symptoms of hypophyseal neoplasia in 3 (cases 10, 16, and 19). In one of these (case 19) bilateral testicular atrophy was caused by mumps orchitis; in the other 2 the etiologic factors were unknown. In 5 patients (cases 9, 10, 16, 18, and 20) functional or anatomical evidence of gonadal deficiency developed during the course of the illness but exact time relationships could not be determined. In 3 patients (cases 11, 12, and 14) normal libido was present until death. No data were available on the testicular function of patient 13. In one patient (case 10) hot flashes were an early symptom. Gonadotropin was absent in the urine of 2 patients, but determinations were done late in the course of the disease (cases 10 and 19). Gonadotropin excretion was normal in 2 men (cases 11 and 12).

Thyroid Gland

Somatic Overgrowth. At necropsy, the thyroid glands were nodular in 3 patients (cases 1, 4, and 6), fibrosed in one (case 5), and normal in 2 (cases 2 and 8).

Thyroid function varied widely; one patient (case 5) developed myxedema after acromegaly had become established, and another (case 6) developed fulminating thyrotoxicosis. Six patients appeared to be euthyroid.

"Chromophobe Adenomas." The thyroid glands were small or fibrosed in 5 patients (cases 10, 12, 16, 19, and 22). In 3 they were nodular or unusually large (cases 9, 18, and 25). In the remainder they were within normal anatomical limits.

One patient had clinical myxedema which, it is of note, preceded the manifestations of hypophyseal neoplasia (case 12). The basal metabolic rate was abnormally low in 3 other patients (cases 10, 16, and 17), but was not obtained until after sellar symptoms were manifest.

Pancreas

Somatic Overgrowth. The islands of Langerhans appeared histologically normal in all but one patient (case 8), in whom they were hyperplastic. Four of the 6 acromegalic patients whose island function was investigated had either manifest or occult diabetes (cases 2, 4, 5, and 6).

"Chromophobe Adenomas." Sections of pancreas were available from 15 patients with "chromophobe adenomas." Islands were un-

usually large or abundant in 9 (cases 9, 10, 11, 14, 20, 24, 25, 26, and 27). Four of 9 patients investigated clinically had manifest diabetes mellitus (cases 14, 16, 18, and 22). Blood sugar was normal in 5 patients (cases 10, 11, 12, 15, and 19).

Parathyroid Glands

Somatic Overgrowth. There was hyperplasia of the parathyroid glands by the anatomical criteria of Castleman and Mallory[9] in 4 of 5 patients from whom they were available, although none had clinical signs of hyperparathyroidism.

"Chromophobe Adenomas." Parathyroid glands of 8 of 19 patients with "chromophobe adenomas" were examined. Three cases showed adenoma, hyperplasia, or both (cases 20, 25, and 27). One of the 3 (case 25) had clinical hyperparathyroidism of 30 years' duration.

Endometrium

Somatic Overgrowth. The endometrium of 3 women was examined. One had a large endometrial polyp with atrophic ovaries (case 5), another had atrophic endometrium with hyperplastic ovaries (case 6), and a third had normal proliferative endometrium with one normal ovary (case 8).

"Chromophobe Adenomas." The endometrium was studied in 2 patients with "chromophobe adenomas." One (case 24) had endometrial and cervical polyps with marked stromal hyperplasia of the ovaries; the other (case 26), an 80-year-old woman, had marked glandular hyperplasia in the presence of totally atrophic ovaries.

Breast

Somatic Overgrowth. There was mammary stimulation in all of the 4 women from whom breast tissue was submitted for examination. In patient 2, a 25-year-old nulligravida, this had progressed to active lactation.

"Chromophobe Adenomas." The breasts of 6 of the 7 women with "chromophobe adenomas" showed anatomical or functional abnormalities. There was secretory activity in 3 patients past 40 years (cases 22, 23, and 25). Patient 21 was operated upon for mastopathia cystica, while patients 24 and 26 had carcinomas of the breast. The breasts of the seventh woman were not examined. Secretory activity and intraductal hyperplasia characterized the single male breast studied (case 11).

Associated Extra-Endocrine Tumors

Somatic Overgrowth. Five individuals of this series had a total of eleven extra-endocrine tumors as follows: carcinoma of the colon, acute myelogenous leukemia, polyp of the ileum, meningioma, neuroma, lipomas (2 patients), multiple skin fibromas, osteoma, hemangioma, and cholangioma.

"Chromophobe Adenomas." There were thirteen extra-endocrine tumors in seven individuals with "chromophobe adenomas," as follows: carcinoma of the colon, carcinoma of the breast (2 patients), carcinoma of the bladder, polyposis of the colon (2 patients), polyposis of the stomach, fibromas of the skin (2 patients), multiple skin papillomas, leiomyoma of the stomach, osteoma, and cystadenoma of the pancreas.

Discussion

For many years, the origin of most, if not all, of the tropic hormones has been ascribed to the "acidophils."[10,11] This hypothesis was based on the histologic appearance of the hypophysis stained with trichrome techniques. Ever since the introduction of the PAS technique, however, emphasis has shifted to the mucoprotein-containing "basophils" because of the increase of Schiff-positive elements in the hypophysis associated with increased activity.[12,13] It seems possible that both schools actually described the same cell, which is here referred to as an "amphophil" and which, as noted previously,[6] can be stained either red or blue by trichrome methods.

The hypophyseal adenomas of both the "acidophil" and "chomophobe" varieties, as here reported, are composed predominantly of amphophils. These cells have been implicated in the production of tropic hormones acting upon various target organs.[2,3,5,14] Animal experiments, as well as clinical observations in man, suggest that deficiency in an endocrine target organ may cause hypophyseal hyperplasia or neoplasia together with increased hypophyseal secretion stimulating in turn the deficient target gland or, occasionally, other endocrine organs.

Gonadal Deficiency. In some strains of mice, early gonadectomy is followed commonly by hypophyseal hyperplasia of "basophil cells resembling hypertrophic amphophils."[15] In addition, the adrenal glands and the breasts react with hyperplasia or even carcinoma. These experimental observations may parallel those of the patients of this report whose hypophyseal tumors were associated with early gonadal failure, adrenal hyperplasia, and mammary hyperplasia or carcinoma.

The increased height and acromegalic features of eunuchs are old folk observations. The concurrence of hypophyseal tumors and castrate acromegaly has been documented in the Skopecs of eastern Europe, a religious sect practicing ritual castration.[16] In canine[17] and in human[18] surgical castrates, hyperplasia of "large chromophobes" or "unripe acidophils" has been reported. Judging by the illustrations, these designations refer to cells here called "amphophils."

Thyroid Deficiency. The association of thyroid deficiency with hypophyseal enlargement in man[19] and animals[17] has been known for a long time. By destroying thyroid glands with I^{131}, hypophyseal tumors which secrete thyrotropin and, possibly, small amounts of gonadotropin have been produced in mice.[20] By the same sequence, thyroid failure may have initiated hypophyseal neoplasia in case 12 of this report.

Adrenal Deficiency. In animals, "chromophobe" hyperplasia can be induced by the agency of relative adrenal insufficiency incident to prolonged stress.[21] Enlargement of the hypophysis in association with hyperplasia and mitotic activity of amphophils has been reported in patients with Addison's disease.[2] "Chromophobe adenomas" of microscopic dimensions are exceedingly common in the hypophyses of patients dying after long illnesses,[22] presumably the result of a stressing mechanism. Although overt adrenal insufficiency was not demonstrated in any of our patients, relative hypocorticism might have operated in the pathogenesis of some hypophyseal tumors, particularly in those patients in whom no other target organ deficiency was found.

Irradiation. Hypophyseal tumors stimulating the gonads, adrenal and thyroid glands, breasts, and somatic growth have been observed following whole body irradiation in various strains of mice.[23] It is not known whether irradiation produces this effect through non-specific stress or through gonadal damage. Patient 23 of this report had received a course of spray radiation for presumed Hodgkin's disease 20 years before the onset of her hypophyseal tumor. Secretion could be expressed from her breasts. No data are available on other target organs.

Estrogens. The development of hypophyseal hyperplasia and tumors by means of estrogenic stimulation constitutes a mechanism which is not only different from the pathogenic sequence just outlined but also one which seemingly is operative only in animals. Thus in rats[24] and mice[25] prolonged estrogen treatment will result in the formation of "chromophobe adenomas." In striking contrast, the hypophyses of women given estrogens for long periods tend to be smaller than nor-

mal and the amphophil series of cells is diminished.[4] It is possible that in animals, some phenomena commonly attributed to estrogen treatment per se, such as adrenal hyperplasia,[26] Leydig cell hyperplasia and tumors,[27] and mammary carcinomas,[25,27] are in fact mediated through the amphophils.

There was predominance of amphophils both in the patients of our series with somatic overgrowth and in the patients with "chromophobe adenomas." This, together with the associated anatomical and clinical observations, suggests that the amphophils are capable of producing growth hormone, ACTH, gonadotropin, thyrotropin, and mammotropin. However, it is not implied that these hormones are all produced either simultaneously or by all tumors. The amphophils, it appears, have some direct or indirect relation to diabetes, to hyperplasia of the islands of Langerhans, and to hyperplasia of the parathyroid glands. The difference between the hypophyseal tumors of acromegalic and non-acromegalic patients seems to be quantitative rather than qualitative. It also appears that some of the symptoms of patients with "chromophobe adenomas" are due to hypophyseal hyperfunction rather than to hypophyseal hypofunction or "panhypopituitarism" as usually stated.

Summary

The hypophyses of 7 acromegalic patients, one non-acromegalic woman 185 cm. tall, and 19 patients with so-called chromophobe adenomas were studied histologically. Regardless of the presence or absence of acromegaly, the patients who had not received previous endocrine medication had hypophyseal tumors which were composed of weakly Schiff-positive amphophils rather than Schiff-negative acidophils or chromophobes. Administration of thyroid extract, sex hormones, or adrenal steroids tended to reduce cell size, to produce nuclear pyknosis, and to increase the proportion of acidophils and chromophobes. One patient with classic acromegaly had a nontumorous hypophysis of normal size in which the proportion of amphophils was increased.

Review of clinical and anatomical data with respect to other endocrine glands suggested that gonadal or thyroid failure may have preceded the appearance of the hypophyseal tumor in some patients. Adrenal hyperplasia often was found both with acromegaly and with "chromophobe adenomas." Parathyroid hyperplasia, hyperplasia of the islands of Langerhans, evidence of mammary and endometrial stimulation, and multiple extra-endocrine tumors frequently were

present in both groups of patients. Many patients in both groups had diabetes mellitus.

The hypotheses are advanced, first, that the amphophil cells may secrete growth hormone, ACTH, thyrotropin, gonadotropin, and mammotropin, although not necessarily simultaneously, and, second, that target organ deficiency may be involved in the pathogenesis of some hypophyseal tumors in man.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

All illustrations except Figure 8 were made from sections stained by the periodic acid-Schiff method (PAS) and counterstained with orange G.

FIG. 1. Cell types in the hypophysis of patient 6, acromegaly with no hypophyseal tumor. Amp=sparsely granulated amphophil, HA=hypertrophic amphophil, B=basophil, Chr=chromophobe. × 900.

FIG. 2. Cell types in the hypophysis of patient 6. HyB=basophil showing Crooke's hyaline change, Ac=acidophil, Chr=chromophobe. × 900.

FIG. 3. Invasive amphophil tumor in the hypophysis of patient 3, acromegaly with no recent hormone therapy, no irradiation. There are large, pleomorphic nuclei and prominent nucleoli. × 450.

FIG. 4. High-power view of amphophil tumor, patient 3, to show fine cytoplasmic granulation, nuclear detail, and large vesicular nucleolus. × 972.

FIG. 5. Extrasellar amphophil tumor in the hypophysis of patient 4, acromegaly with no hormone therapy or irradiation. Nuclei are smaller and more uniform than in the tumor illustrated in Figure 3. × 450.

FIG. 6. Intrasellar amphophil adenoma in the hypophysis of patient 1, acromegaly with two courses of irradiation, no recent hormone therapy. Of note are nuclear pleomorphism and similarity to Figure 4 in spite of x-ray therapy. × 972.

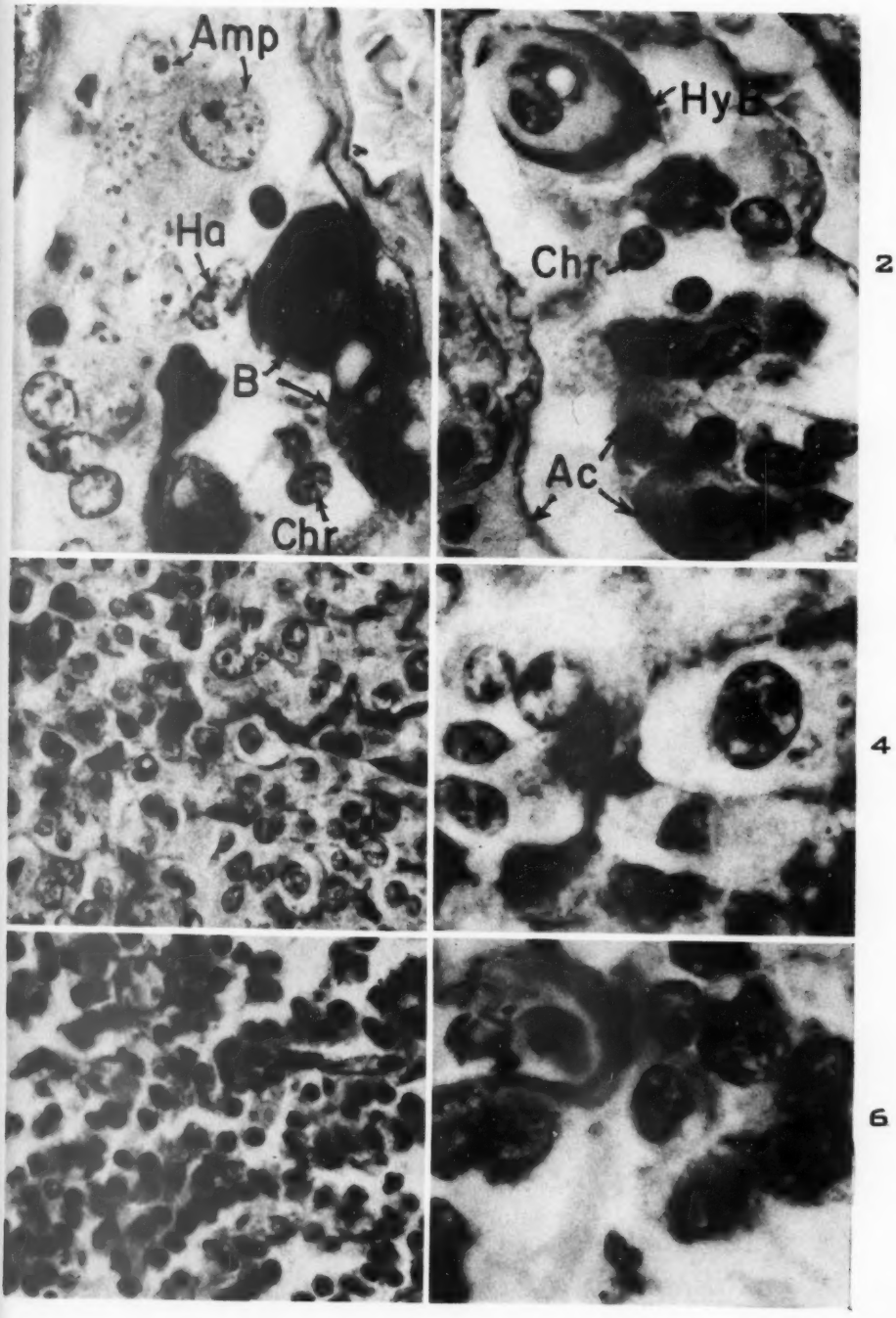
Amp
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FIG. 7. Intrasellar acidophil adenoma in the hypophysis of patient 2, acromegaly treated with estrogens for 6 years, no irradiation. Pyknotic nuclei are small as compared with the tumors shown in Figures 3 and 5. × 450.

FIG. 8. High-power view of acidophil adenoma, patient 2, showing distinct cell boundaries, uniform nuclei, and over-all resemblance to mature acidophils in Figure 2. Hematoxylin and eosin stain. × 972.

FIG. 9. Hypophysis of patient 6, acromegaly without hypophyseal tumor. One course of irradiation; no therapy for 6 years. Without a differential count, this gland could be confused with a normal hypophysis. × 450.

FIG. 10. Intrasellar amphophil adenoma in the hypophysis of patient 24, with a clinical diagnosis of "chromophobe adenoma." No irradiation or hormone therapy. Cells are smaller and more uniform than those of the acromegalic patient shown in Figure 3, but larger and more variable than those of the acromegalic patient shown in Figure 5. × 450.

FIG. 11. High-power view of intrasellar amphophil adenoma of patient 11, with a clinical diagnosis of "chromophobe adenoma." No irradiation. Surgical specimen obtained before terminal hormone therapy. There is a large vesicular nucleolus similar to that found in acromegalic patient 3, Figure 4. × 972.

FIG. 12. Intrasellar chromophobe adenoma in the hypophysis of patient 23. Irradiation and cortisone therapy 3 weeks before surgical removal. Extensive nuclear pyknosis and hydropic cytoplasm may be noted. × 450.

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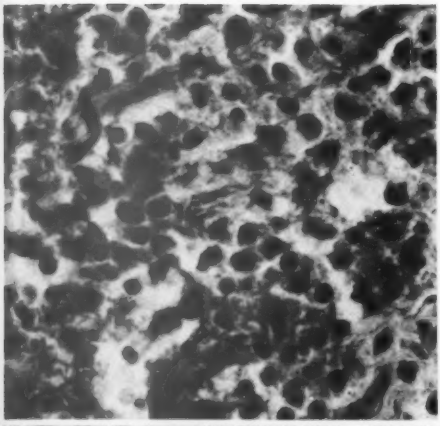

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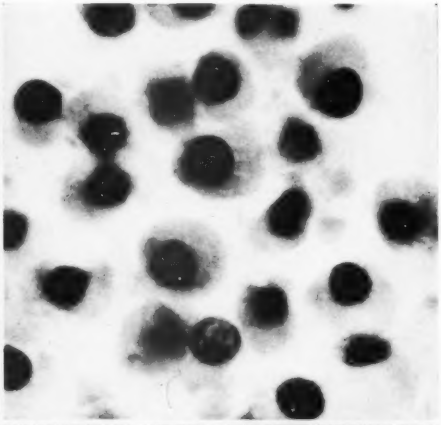

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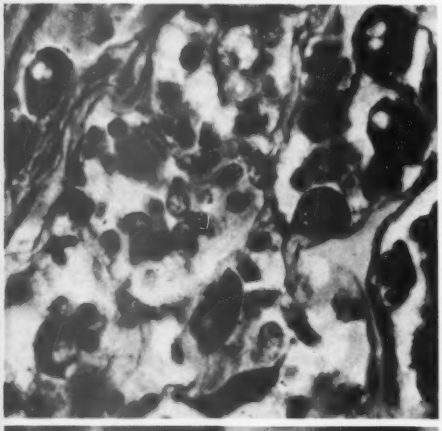

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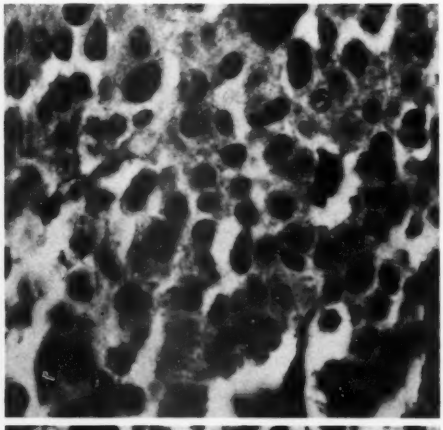

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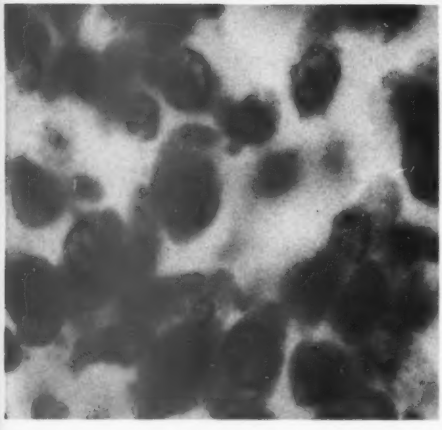

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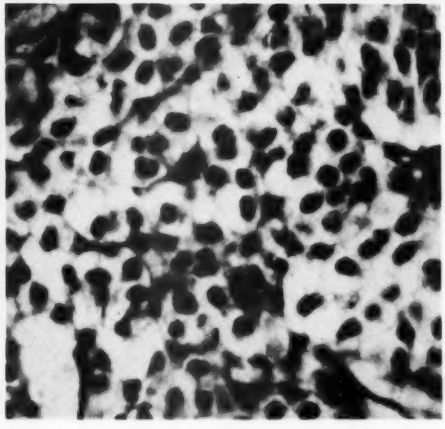

12

FAT NECROSIS OF BONE MARROW IN ACUTE PANCREATITIS*

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Widely disseminated fat necrosis in acute pancreatitis was first reported by Balser[1] in 1882. In his classical description, fat necrosis was present in the mediastinal and pericardial fat in 2 of 5 cases. Other examples of fat necrosis at foci distant from the pancreas have been observed in the subcutaneous tissues by Hansemann,[2] Chiari,[3] and Blauvelt[4]; and in the medulla of long bones in a case reported by Ponfick.[5]

It is generally accepted that fat necrosis in acute pancreatitis usually is localized in the pancreas and adjacent tissues, and only rarely involves extra-abdominal organs. An analysis of 25 necropsied cases of acute pancreatic necrosis by Roberts, Baggenstoss, and Comfort[6] showed pericardial and mediastinal involvement in 12 per cent. The frequency of fat necrosis in bone marrow has not been investigated previously; consequently, this study was undertaken, utilizing both experimental and clinical material.

MATERIALS AND METHODS

Experimental Animals

Six healthy male mongrel dogs, weighing 10 to 20 kg., were used in these experiments. Acute hemorrhagic pancreatitis was produced by surgical separation of the pancreas from the duodenum, thus depriving it of its blood supply. These animals were necropsied within 1 hour after death. The femora, humeri, sternum, and several ribs were removed.

Multiple step sections were cut at various levels from paraffin-embedded material and were stained routinely with hematoxylin and eosin. In addition, positive and suspicious lesions were stained by the von Kossa[7] and Fischler[8,9] techniques for the demonstration of calcium soaps.

Human Material

Human bone marrow was obtained from both necropsy material and clinical cases of acute and subacute pancreatitis. Our necropsy material consisted of 67 cases of acute hemorrhagic pancreatitis which were

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necropsied at the Ohio State University Hospital from August, 1938, to September, 1955. In all but 3 cases, single bone marrow specimens were stained and studied in a manner similar to that used on the experimental material. Six clinical cases of acute and subacute pancreatitis were studied by sternal bone marrow aspiration. In 2 cases two aspirations were performed 4 weeks apart. Supravital preparations, Wright's stained smears, and paraffin blocks were prepared from the sternal aspirates.

Results

Fat necrosis of bone marrow was found in 7 (10.4 per cent) of the 67 necropsied cases (Table I). Although all 6 dogs expired with acute pancreatic necrosis, bone marrow lesions were found in only 2 animals. The majority of lesions in the experimental and human cases were

Table I

Incidence of Fat Necrosis of Bone Marrow in Human Cases of Acute Pancreatitis

Gross pathologic features	No. of cases	Incidence of fat necrosis in bone marrow	Percentage
Acute hemorrhagic pancreatitis without fat necrosis	29	0	
Acute pancreatitis with focal fat necrosis	23	0	
Acute pancreatitis with widespread abdominal fat necrosis	12	4	33.3
Acute pancreatitis with extra-abdominal fat necrosis	3	3	100.0
	67	7	10.4

found in bone marrow of the ribs. Lesions involving more than one bone were found in one dog and in 2 human cases in which several bones were studied.

It is of interest that bone marrow lesions were found only in cases of acute pancreatitis with either widespread abdominal or extra-abdominal fat necrosis. No relationship was found to exist between the presence of bone marrow lesions and the serum amylase level or duration of disease (Table II).

The bone marrow lesions varied greatly in size. The largest lesion measured approximately 2 mm. in diameter while the smallest was demonstrable only under high magnification. Although the majority of lesions were classical examples of fat necrosis, considerable morphologic variation was noted in the early and late stages of necrosis. The structure appeared to be related not only to the age of the lesion but also to the amount of calcium soaps present and the degree of calcifi-

cation. The earliest lesion consisted of a small, circumscribed focus of necrotic cells showing pyknosis and loss of cellular outline (Fig. 1). Another lesion representing a later stage showed necrosis of a small cluster of fat cells with capillary congestion and numerous phagocytes displaying a granular and vacuolated cytoplasm (Fig. 2). Several lesions showed typical islands of fat necrosis in which the fat cells had

TABLE II

Analysis of 2 Experimental and 7 Human Cases of Fat Necrosis of Bone Marrow in Acute Pancreatitis

Case	Clinical data			Days with disease	Serum amylase units/100 cc.	Site of lesion	Type of lesion
Dog 1	Surgical separation of pancreas from duodenum			3	2560	Rib	Early necrosis (Fig. 1)
Dog 5				8	680	Rib, femur	Focal fat necrosis
	Age	*Race*	*Sex*				
A-12552	46	W	M	30	25, 45	Rib	Highly calcified lesion (Fig. 7)
A-16399	74	W	F	7	Not tested	Vertebra	Focal fat necrosis with epithelioid cell response (Fig. 5)
A-19659	49	W	M	3	1222	Vertebra	Early fat necrosis with phagocytic infiltration (Fig. 2)
A-19847	68	N	F	27	11, 23	Rib	Fat necrosis (lesion 2 mm. in diameter, Fig. 3)
A-19957	72	W	F	6	469	Rib, vertebra	Focal fat necrosis
A-20041	32	N	M	4	302	Rib, vertebra	Focal fat necrosis with early calcification (Fig. 4)
A-20069	48	W	F	5	451	Rib	Focal fat necrosis

lost their nuclei but retained their cellular configuration (Fig. 3). When stained by the von Kossa method, calcium soaps appeared as black granules (Fig. 4). These lesions were surrounded by a margin of leukocytes; in one case a foreign body reaction was present, as characterized by groups of epithelioid cells (Fig. 5). One suspicious lesion (Fig. 6) stained by the Fischler technique showed formation of the black hematoxylin lake characteristic of calcium soaps (Fig. 7).

In the 6 clinical cases no lesions could be demonstrated by bone marrow aspiration.

DISCUSSION

A wide variety of lesions involving numerous organs have been reported in acute pancreatitis: muscle hematoma,[10] capillary necrosis with subependymal gliosis of brain,[11] and focal necrosis of heart, adrenal glands, and ovaries with demyelination of the brain.[12] It is believed that these lesions are the result of high levels of circulating

enzymes liberated by the damaged pancreas. Although a search of the literature has uncovered only one report,[5] fat necrosis of the bone marrow in acute pancreatitis seems by no means a rare lesion. A careful search of multiple bone marrows, with histochemical investigation of suspicious lesions, has resulted in the demonstration of fat necrosis in 10.4 per cent of our cases. Its frequency appears to be dependent on the presence of widespread abdominal or extra-abdominal fat necrosis.

The dissemination of lipase in acute pancreatitis had been demonstrated by Rostock[13] and Perry[14] to take place primarily through the lymphatics, although hematogenous spread also may occur. Abdominal peritoneal lymphatic drainage[15] and transdiaphragmatic lymphatic absorption of abdominal fluid[16] are probably prime factors in the ingress of pancreatic lipase into the thoracic duct and thence into the general venous circulation. This may account for the close relationship between widespread abdominal fat necrosis and bone marrow involvement.

The pathophysiologic implications of an enzymatic lesion of the bone marrow are obvious. The profound hemodynamic alterations consequent to shock in acute hemorrhagic pancreatitis and the ensuing intravenous therapy made it impossible to ascertain, by a review of peripheral blood counts, whether a depression of erythropoiesis and myelopoiesis was present. However, because of the focal nature of the lesion, a significant depressive effect on hematopoiesis is unlikely and was not observed in our cases. Although no extensive clinical studies were done, the diagnostic value of bone marrow aspiration in acute pancreatitis appears to be limited because there is no constant localization of the lesions in any one bone. However, the demonstration of bone marrow fat necrosis by sternal aspiration *in vivo* should indicate the presence of widespread abdominal fat necrosis and consequently influence the prognosis.

Summary

The bone marrows of 67 necropsied cases of acute hemorrhagic pancreatitis were examined for evidence of fat necrosis. In 7 cases lesions were found ranging from early necrosis to heavily calcified areas in which calcium soaps could be identified, although the typical structure of fat necrosis was absent.

In 2 of 6 dogs dying of acute hemorrhagic pancreatitis, fat necrosis was present in the bone marrow.

In both the experimental and human cases, bone marrow lesions were found only in the presence of either widespread abdominal or extra-

abdominal fat necrosis. The pathogenesis of bone marrow fat necrosis is discussed in relation to lipase dissemination via the peritoneal and transdiaphragmatic lymphatics.

Because of the focal nature of bone marrow fat necrosis, its pathophysiologic and diagnostic importance is probably very limited.

I am grateful to Dr. Robert M. Zollinger, Professor of Surgery at the Ohio State University College of Medicine, for allowing me to study his clinical cases of pancreatitis and to Dr. Roger D. Williams, Assistant Professor of Surgery, for supplying dogs with acute pancreatitis. The photographs are the work of Mr. Gilford Millard.

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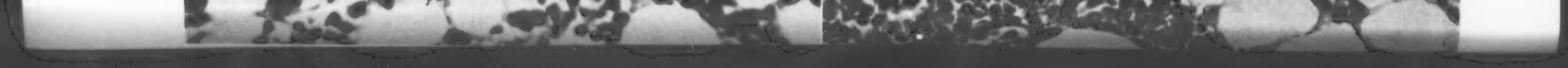
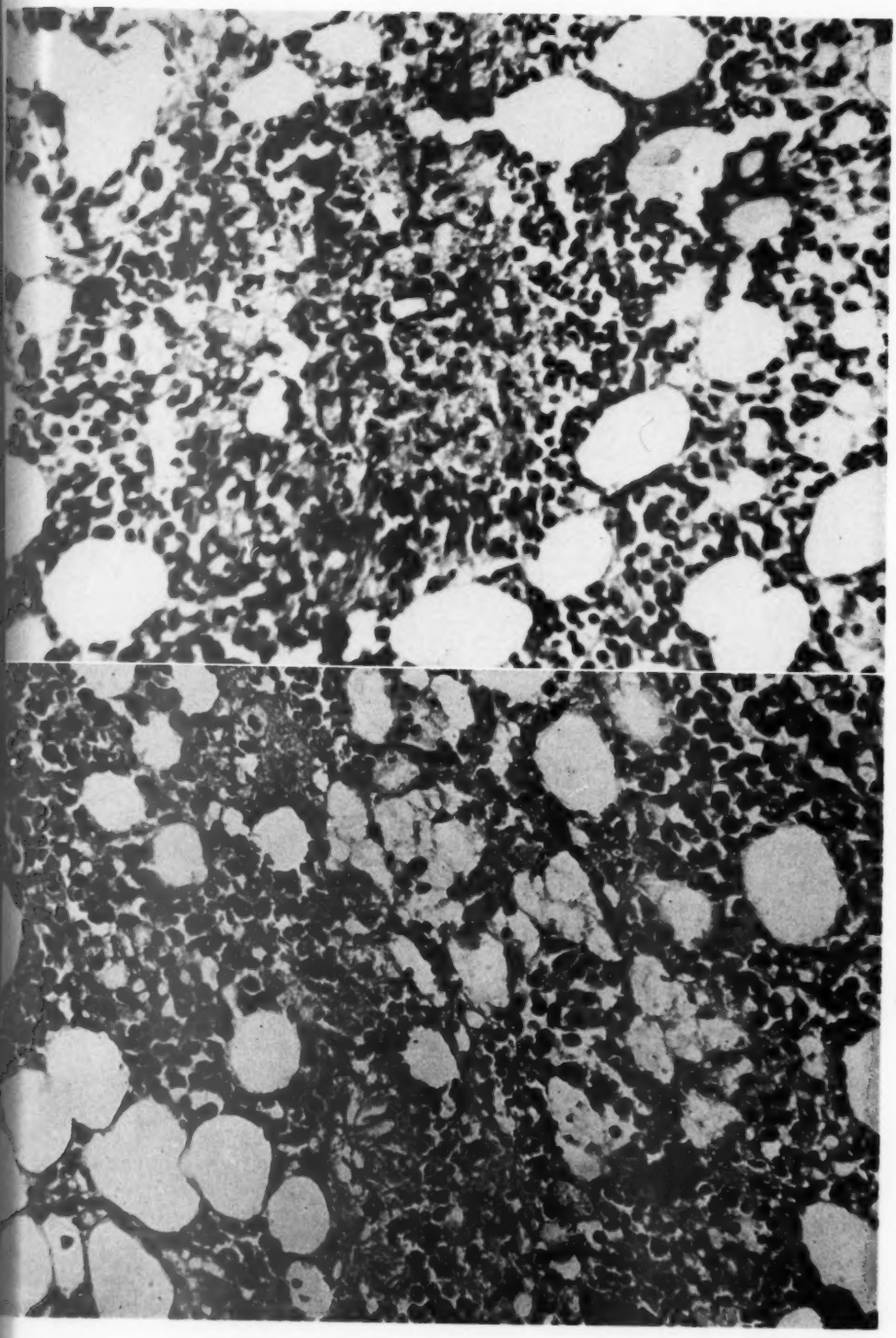
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Early focal necrosis of bone marrow, with pyknosis and loss of cellular outline. Hematoxylin and eosin stain. × 305.

FIG. 2. Focal fat necrosis of bone marrow with a central cluster of phagocytes. There is capillary congestion at the periphery of the lesion. Hematoxylin and eosin stain. × 315.





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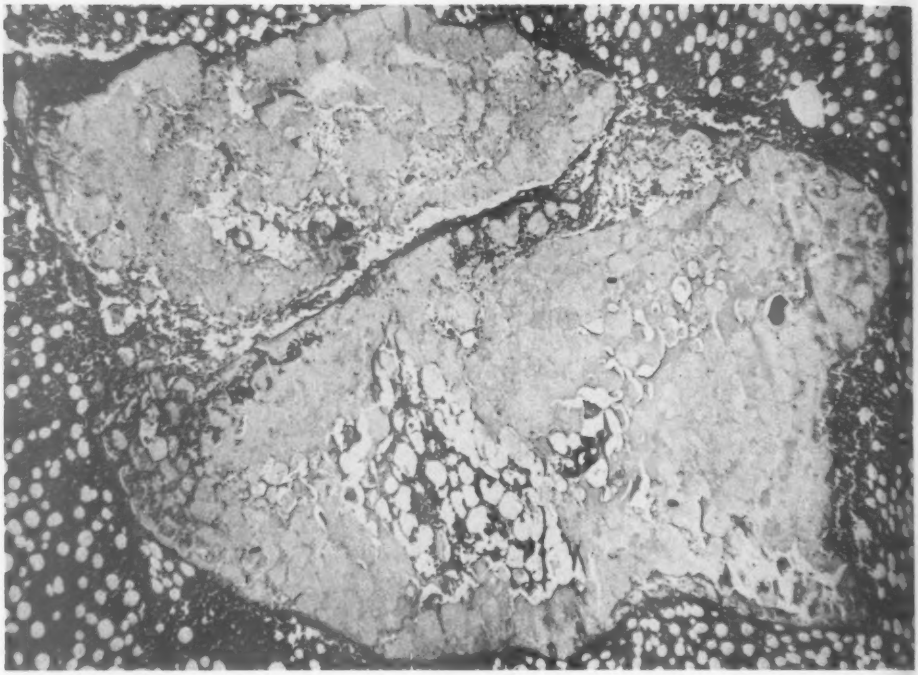
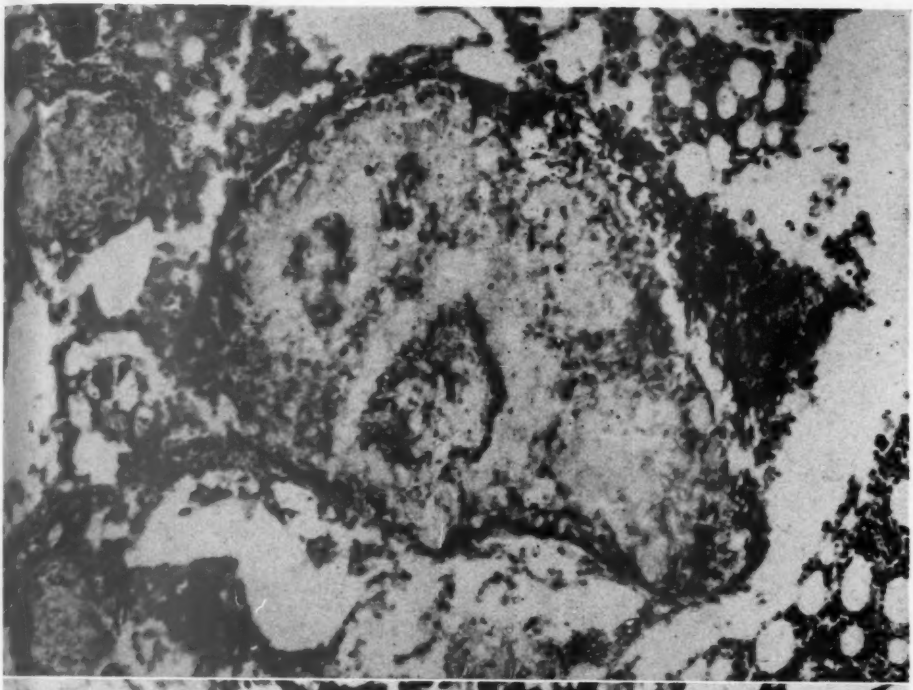


FIG. 3. A large focus of fat necrosis. The necrotic fat cells have lost their nuclei but have retained their circular outline. A margin of leukocytes surrounds the lesion. Several islands of bone marrow are imprisoned within the lesion. Hematoxylin and eosin stain. × 16.

FIG. 4. An area of fat necrosis showing the black granular appearance of calcium soaps. The soaps appear to be concentrated at the periphery of necrotic fat cells. Von Kossa's silver technique with safranine counterstain. × 110.

FIG. 5. Bone marrow adjacent to an area of fat necrosis, showing early necrosis and a foreign body reaction characterized by a group of epithelioid cells. Hematoxylin and eosin stain. × 180.



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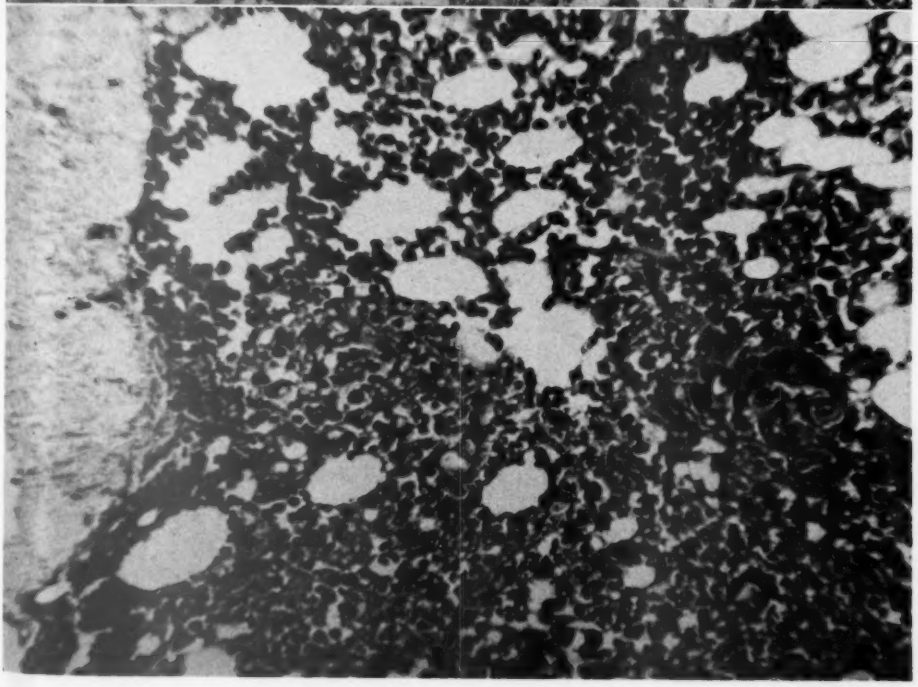

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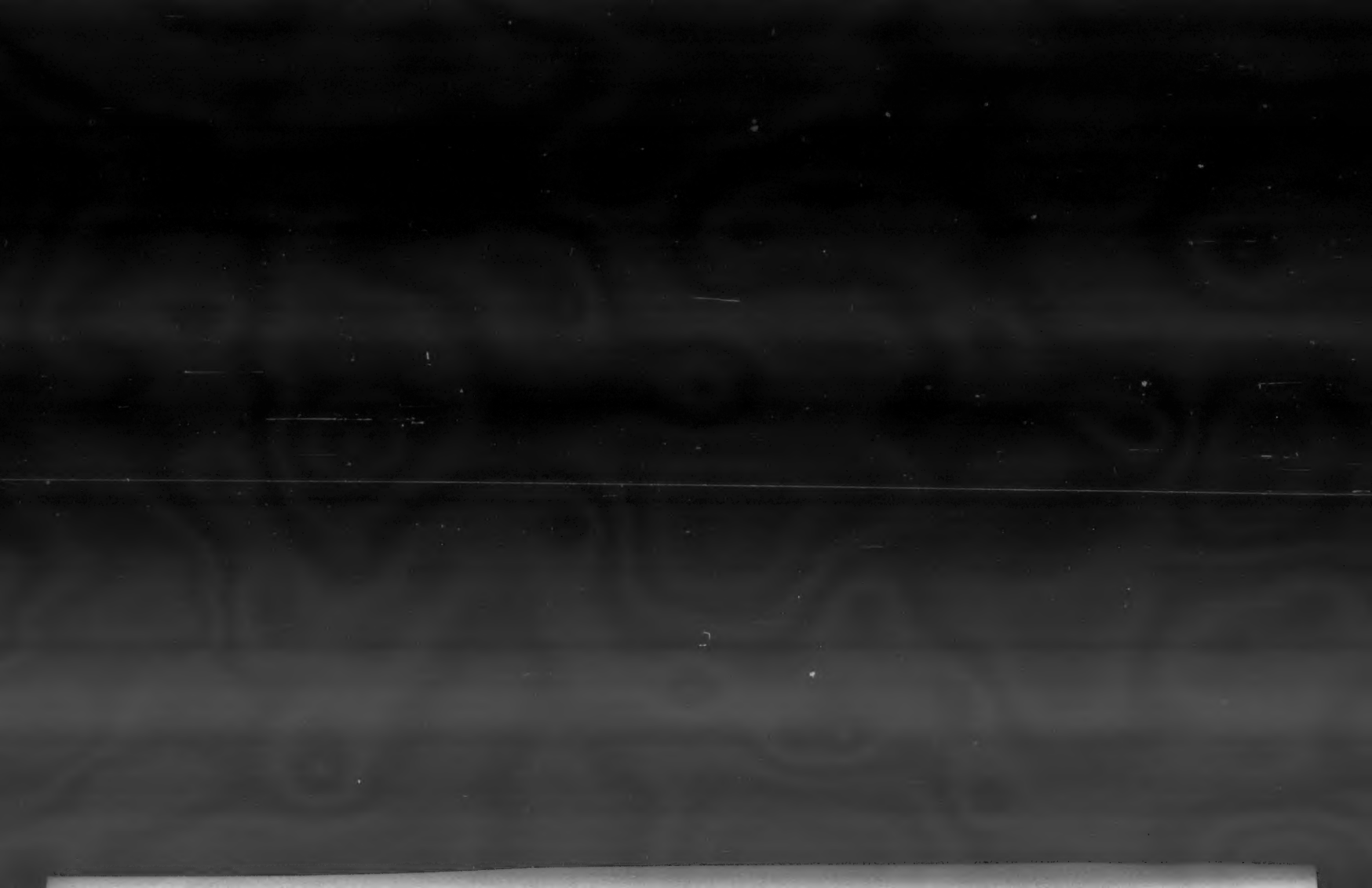
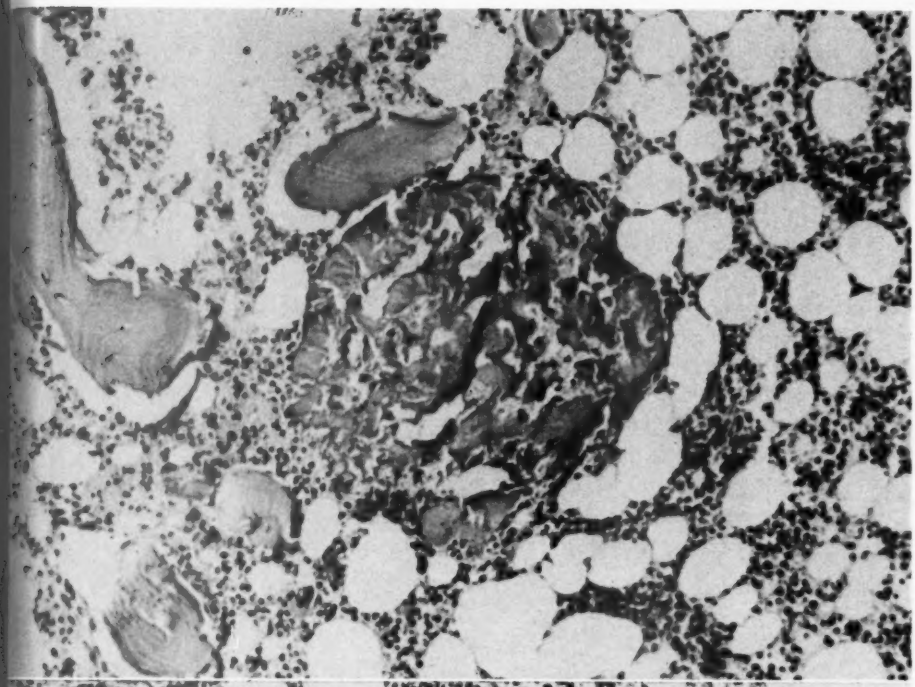
FIG. 6. An old bone marrow lesion which has lost the characteristic appearance of fat necrosis. Of note also is the absence of inflammation. Hematoxylin and eosin stain. × 160.

FIG. 7. A serial section of the lesion shown in Figure 6, showing the presence of calcium soaps and fatty acids. Fischler technique with safranine counterstain. × 160.





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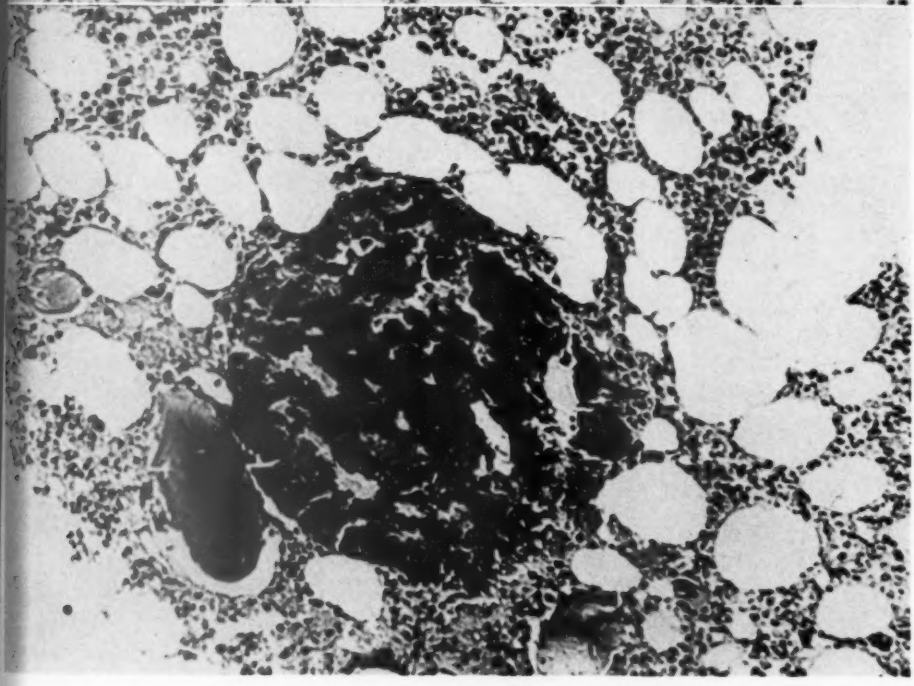

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